STUDIES ON THE DISTRIBUTION OF THE ALLOTYPIC VARIANTS OF THE IgG RECEPTORS (FcγRIIa AND FcγRIIIb) AND THEIR ASSOCIATION WITH SEVERE CLINICAL MALARIA AMONG GHANAIAN CHILDREN

A THESIS SUBMITTED

BY

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B. Sc. (University of Science and Technology), 1993.

TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF SCIENCE, UNIVERSITY OF GHANA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE MASTER OF PHILOSOPHY DEGREE IN BIOCHEMISTRY

SEPTEMBER 2001

DECLARATION

I hereby declare that, with the exception of references to other peoples work which I have duly acknowledged, all the experimental work described in this thesis was carried out by me, and this thesis, either in whole or in part has not been presented elsewhere for another degree.

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To my children; Kimmy, Kobby and Joojo

ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr Y.D. Osei and Dr M.D. Wilson for their guidance, advice and support throughout this project. I am also grateful to Dr D. Adu and Dr W. Tse of the Queen Elizabeth Hospital, Birmingham, UK for their immense contribution in donating primers and giving valuable advice.

The field work done at Dodowa was made possible with the generous help and assistance of Dr K.A. Koram and the staff (Mr Fenteng, Mr Osei, Mr Attiogbe, Mr Boafo and Miss Celestina Opoku-Ampomah) of the Epidemiology Unit of the Noguchi Memorial Institute for Medical Research. The work done at the Department of Child Health, Korle-Bu Teaching Hospital could not have been done without the support of Dr P.O. Rodrigues, the then acting head of the Department, Dr B. Goka, physician in charge of the Malaria-2000 project and her team of physicians, and Dr B.D. Akanmori and staff of the Immunology Unit of the Noguchi Memorial Institute for Medical Research.

I am especially grateful to Mr Addai of the Haematology Unit of the Noguchi Memorial Institute for Medical Research and Mr Ankrah of the Clinical Pathology department of the Korle-Bu Teaching Hospital for helping in the laboratory analysis of the blood samples. I would also like to acknowledge the tremendous help and goodwill received from the workers and students of Parasitology Unit of the Institute. I am particularly grateful to Dr Daniel Boakye, Mr C.A. Brown, Mrs Anita Ghansah, Ms Nancy Duah, Ms Rita Osei, Ms Helena Baidoo, and Ms Naikki Pupulampu for their immense encouragement and support.

I would very much like to express my gratitude to the staff of the Department of Biochemistry, University of Ghana, Legon without whom this work would not have been possible.

My utmost gratitude goes to my dear husband for providing the necessary funds for this project work, my mum, my siblings and my pastor Bishop Dag Heward Mills and his family for all their support. Above all, this would not have been possible without the Lord Almighty, and I give him all the glory.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ADCC	Antibody dependent cellular cytotoxicity
ANCA	Anti-neutrophil cytoplasmic antibodies
ARDS	Adult respiratory distress syndrome
BSM	Bispecific molecules
F ab	Fragment antigen binding
Fc	Fragment crystallisable
GPI	Glycosylphosphatidylinositol
h	Human
HR	High responders
IC	Immune complexes
Ig	Immunoglobulin
LR	Low responders
m	Murine
mAB	Murine antibody
MHC	Major histocompatibility complex
NA	Neutrophil antigen
NK	Natural killer cells
PMN	Polymorphonuclear cells
RBC	Red blood cells
RIA	Radioimmunoassay
SDS	Sodium dodecyl sulphate
SLE	Systemic lupus erythematosus
TNF	Tumour necrosis factor

LIST OF APPENDICES

ABSTRACT

The immunoglobulin G (IgG) receptors FcγRIIa and FcγRIIIb vary among different ethnic groups, and more importantly, are also known to be associated with either susceptibility to or protection from certain diseases. These variations are manifest as differences in the antigen-binding capabilities of the receptors. The genes encoding for these receptors are polymorphic and these also vary among populations. These variations can be investigated using molecular methods such as PCR. The aim of the present study was to determine if there were any significant differences in the distribution of these genotypes among some of the ethnic groups in Ghana and also if there was any association between the genotypes and the incidence of severe malaria.

A total number of 329 children, belonging to four different tribes were recruited for the study, of which 75 healthy individuals formed the control group. The 254 patients who were recruited were diagnosed as having uncomplicated, severe anaemia or cerebral malaria. Human DNA was isolated from filter paper blood blots for PCR analysis using allele-specific primers for FcγRIIa to detect H/H131, H/R131, R/R131, and FcγRIIIb to detect NA1/NA1 and NA2/NA2 genotypes.

The results obtained revealed that there was no association between ethnicity and the FcγRIIa genotype, (P=0.78) and FcγRIIIb genotypes (P=0.23). With regard to the incidence of malaria and the FcγRIIa genotypes, the following associations were found ($P \leq 0.002$ in all cases); firstly, there were significantly more of the homozygous FcγRIIa-R/R131 genotypes among patients with severe anaemia and cerebral malaria. Secondly, there were significantly less of the homozygous H/H131

in all the three forms of malaria and thirdly, the heterozygous FcγRIIa-H/R131 was associated with only severe anaemia. These observations suggest that the homozygosity of the R allele is a heritable risk factor for severe malaria, while the H allele confers some degree of protection against the disease both in the homozygous and heterozygous state.

Similarly, no association was found between the FcγRIIIb and ethnicity ($P>0.05$. The homozygous NA1/NA1 was found to be significantly dominant among the patient group ($P=0.003$). The NA2/NA2 genotype on the other hand was significantly reduced in the patient group ($P=0.000$), and also in all the three forms of the diseases ($P< 0.04$). The heterozygous NA1/NA2 was found to be intermediate between the two, but was significantly underepresented in the cerebral and severe anaemia patients ($P\leq 0.001$). The null genotype was found to be overepresented within the patient group ($P=0.02$).

These findings suggest that the NA2 offers protection against all forms of malaria and this effect is even observed in the heterozygotes NA1/NA2 among the severe anaemia and cerebral malaria groups. NA1, on the other hand is a heritable predisposing factor to symptomatic malaria.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Human immunoglobulin G receptors (FcγR) provide an important link between the humoral and cellular branches of the immune response. FcγR engagement may result in a plethora of biological responses including phagocytosis, endocytosis, antibody-dependent cellular cytotoxicity (ADCC), release of inflammatory mediators, facilitation of antigen presentation and clearance of immune complexes (Van de Winkel & Capel, 1996).

FcγR belongs to the Immunoglobulin (Ig) super gene family and three classes of leukocyte FcγR encoding 12 receptor isoforms have so far been identified (Van de Winkel and Anderson, 1991). These expressed molecules differ in their molecular weights, affinity and specificity for Immunoglobulin G (IgG) isotypes, cell distribution patterns and capacity to trigger intracellular signals (Kimberly *et al.*, 1995).

Fcγ receptors participate in a much broader range of functions other than disposal of immune complexes. For the phagocyte, activation of Fcγ receptors can trigger a spectrum of integrated cell programs including an oxidative burst, secretion of intracellular granule contents, cellular cytotoxicity, gene activation with new protein synthesis and elaboration of various cytokines (Kimberly *et al.*, 1995). Fcγ receptors on B lymphocytes can provide negative regulatory signals, and in the context of autoimmunity, modulation of B cell hyperactivity stands out as a potential

therapeutic target. Fcγ receptors on natural killer (NK) cells mediate antibody-dependent cellular cytotoxicity.

Fcγ receptors, expressed on a broad range of blood cells, play an important role in the binding and phagocytosis of IgG –opsonized particles (Van de Winkel & Anderson, 1991). The most abundant phagocyte, the polymorphonuclear leukocyte (PMN), expresses constitutively two of the three FcγR subclasses, FcγRIIa (CD32) and FcγRIIIb (CD16). FcγRI (CD64) expressed on activated PMN, binds monomeric IgG with high activity. FcγRIIa and IIIb are low affinity receptors interacting with complexed or aggregated IgG.

Both FcγRIIa and IIIb exhibit a polymorphism, which is genetically determined. FcγRIIa may occur as either FcγRIIa-H131 or FcγRIIa-R131 allotype. The substitution of just one amino acid, histidine or arginine at position 131 of the protein molecule accounts for this, and has been found to be critical for IgG binding (Salmon *et al.,* 1992). FcγRIIIb polymorphism can occur either as neutrophil antigen1 (NA1) or neutrophil antigen2 (NA2), and these differ in the number of glycosylation sites at position 65 and 82 (Salmon *et al.,* 1992).

IgG subclass antibodies also have different properties with respect to binding to the IgG receptors bound on the surface of leukocytes (Burton & Woof, 1992). Human IgG1 and IgG3 subclasses interact with all FcγR classes. FcγRIIa has been shown to be the sole FcγR class capable of binding human IgG2, with the H allele having a much higher affinity for IgG2 than the R allele (Salmon *et al,* 1992). With respect to the FcγRIIIb poymorphism, it has been shown that there is a functional difference in

the activity of PMNs expressing the IIIb-NA1 or IIIb-NA2 allotypic forms (Salmon *et al,* 1992).

Results from some studies seem to suggest that the Fcγ receptor polymorphisms may be relevant in health and diseases, especially in situations where human IgG2 is the predominant antibody subclass produced, such as those involving anti-carbohydrate immune responses (Insel & Anderson, 1988). Abnormal Fcγ receptor-mediated clearance of IgG-opsonized erythrocytes, a model for circulating immune complexes, has been demonstrated in systemic lupus erythematosus (SLE) and other immune complex diseases (Frank *et al.,* 1979). These polymorphisms have also been implicated in the susceptibility of humans to heparin-induced thrombocytopenia (Cines *et al.,* 1980; Chong *et al.,* 1982). This is a disorder associated with anti-platelet heparin- dependent antibodies that cluster and trigger the platelet FcγRII (Kelton *et al.,* 1988; Chong *et al.,* 1989). Furthermore, the frequency of individuals homozygous for H131 appears underrepresented in a group of patients with recurrent bacterial infections (Sanders *et al,* 1994), and in a group susceptible to meningococcal disease (Fijien *et al.,* 1993). Also, the level of circulating human IgG2 is significantly lower in H/H131 individuals as compared to those with the R/R131 genotype (Parren *et al.,* 1992).

The frequency of this allotypic polymorphism may vary among ethnic groups. It is possible that ethnic variation in the FcγRIIa allotype frequency may have consequences for the susceptibility, within certain populations, to specific diseases, especially those associated with an human IgG2 humoral response. Reports of high affinity binding of human IgG2 by H131 transfectants (Warmerdam *et al.,* 1991) as well as greater phagocytosis of particles opsonized with human IgG2 by H/H131

PMNs (Salmon *et al.,* 1992) suggest that the H/H131 genotype may have advantages in this respect.

Eastern Asians (Japanese, Chinese, Indians) are more frequently of the murine IgG1 low responder phenotype compared to Caucasians. Such variation may in part explain racial differences in the susceptibility to certain diseases such as those caused by *Haemophilus influenzae* and *Neisseria* (Nagata *et al.*, 1989; Musser *et al.*, 1990; Figueroa & Densen, 1991). In addition to the above, epidemiologic reports also suggest the possibility of such correlations. For example the incidence of meningococcal disease among Japanese is very low compared to non-Japanese inhabitants of the Midwestern United States (Sclech *et al.,* 1985; Densen *et al.,* 1990; Wenger *et al.,* 1990). It has also been reported that *H influenzae* infections are very rare in Japan. In healthy Caucasian donors, the RIIIb-NA2/NA2 phenotype is expressed more often whereas the RIIIb-NA1/NA1 phenotype predominates in Japanese individuals (Ohto & Matsuo, 1989; Sanders *et al.,* 1994).

In Ghana, malaria is a major cause of infant morbidity and mortality. The human disease is a protozoan infection of the red blood cells transmitted by the bite of a blood feeding female anopheles mosquito. There are four generally recognized species of malaria parasites in humans: *Plasmodium falciparum, P. malariae, P. vivax* and *P.ovale* (Garnham, 1966).

Malaria can be complicated (severe) or uncomplicated. The clinical features of uncomplicated malaria are common to all four species of *Plasmodium*. The first symptoms are non-specific and resemble influenza. The duration of illness is proportional to the level of immunity and differs between the parasite species. Death

from *P. vivax, P. ovale* or *P.malariae* infections is very rare. On the contrary, falciparum malaria is a potentially lethal infection. The progression to severe disease can be rapid. The major complications of *P. falciparum* infections in children are cerebral malaria, severe anaemia, respiratory distress and hypoglycaemia (WHO, 1990).

Cerebral malaria is defined as unrousable coma (Blantyre coma scale score≤ 2), asexual parasitaemia, and exclusion of other causes of coma. Severe anaemia is defined as haemoglobin level less than 5g/dl and asexual parasitaemia (WHO, 1991). It usually presents as an acute fall in haemoglobin level. Severe anaemia has a peak incidence in children of age around 1 - 2 years and is common in areas with high seasonal transmission than in those with low seasonal transmission (Snow *et al.,* 1994).

Two types of immunity offer resistance to malaria; innate and acquired immunity (Marsh, 1993). Innate or natural resistance to malaria is associated with haemoglobinopathies, major histocompatibility complex (MHC), Duffy blood groups and other RBC variants (Marsh *et al.,* 1995). Acquired immunity to malaria reflects a shift from a relatively non-specific cell mediated response to a highly specific antibody mediated and cytotoxic T cell responses. Different immune responses come into play depending on the stage in the parasite's life cycle. While there is antibody opsonization of the sporozoites for phagocytosis, the immune response to the liver stages of the parasite involves the killing of intracellular parasites by nitric oxide and cytotoxic T cell destruction of infected liver cells (Hill *et al.,* 1991).

Acute malaria is characterized by non-specific polyclonal B-cell activation. There is reduction in circulating T cells with an increase in the γ/δ T- cell subset (Ho *et al.*, 1990), but other T cell proportions are usually normal (Ho and Webster, 1990). Although residents of hyperendemic or holoendemic malarious areas have hypergammaglobulinaemia, most of this antibody is not directed against malaria antigens (White, 1996). In non-immune individuals, the acute antibody response to infection often comprises mostly Immunoglobulin M (IgM) and Immunoglobulin G_2 (IgG_2) isotypes, which are unable to arm cytotoxic cells and thus kill asexual malaria parasites (Bouharoun-Tayoun and Druilhe, 1992).

Although in adults, IgG_2 is often the predominant antibody isotype in the response against polysaccharide antigens, young children exhibit a slow maturation of this isotype. In neonates, there is always a certain level of maternal IgG_2 found in the sera, which is given transplacentally, but this disappears about 5 months after birth (Roitt, 1988).

It has long been established that the host response to Plasmodium infection includes the production of enlarged populations of both peripheral blood monocytes and mature macrophages. In rodent malaria, experiments have shown that both the spleen and the liver remove parasitized erythrocytes more rapidly from the circulation than they remove unaffected cells (Quinn &Wyler, 1979; Dockrell *et al.*, 1980). Furthermore, macrophages from infected animals are more phagocytic for parasitized cells than are normal macrophages; this enhanced phagocytic activity depends on the presence of either opsonins or cytophilic antibodies and also on the degree of macrophage activation (Criswell *et al.*, 1971; Shear *et al.*, 1979).

Protective antibodies inhibit parasite expansion through co-operation with the monocyte-macrophage series by binding to parasitized erythrocytes and activating their Fc receptors (Bouharoun-Tayoun *et al.*, 1990). Although little is known about the nature of this macrophage activation, it seems reasonable to suppose that it includes increased expression of specific surface receptors for the Fc portion of immunoglobulin G (Fcγ receptors), since this would clearly facilitate the phagocytosis of antibody-coated targets (Lee *et al.,* 1989; Ho *et al.,* 1990).

There are presently three methods that can be used to investigate FcγRIIa polymorphism; these are, (i) functional assays, (ii) immunofluorescence assays using both a pan-CD32 mAb and mAb 41H16 that binds specifically to the FcγRIIa-R131 allotype (Gosselin *et al,* 1990) and (iii) PCR-based methods. There are also methods that can be used to type FcγRIIIb polymorphism and these are (i) biochemical methods, (ii) immunofluorescence assays and (iii) sensitive PCR-based methods.

1.1.1 Rationale

There has been considerable increase in interest in assessing the role of Fcγ receptors because of their importance in providing a bridge between the humoral and cellular arms of the human immune response (van de Winkel and Capel, 1993). Appreciable differences in the distribution of FcγRIIa and FcγRIIIb allotypes have been reported among different ethnic groups. This poses interesting questions about the selective pressure that maintains the polymorphisms in these genes in human populations, and about the impact of this polymorphism in the outcome of infection and clinical manifestations.

Several hospital-based studies have shown an association between FcγRIIa-H/H131 allotype and protection against encapsulated bacterial infections whereas the poorly IgG_2-binding allotype, FcγRIIa-R/R131 is associated with increased susceptibility to these pathogens. No association has so far been reported between any protective immune response and FcγRIIIb allotypes (Van Schie and Wilson, 1997).

Because of the high rate of mortality associated with falciparum malaria especially among children under 5 years of age, it expected that any genetic trait that provides some protection would be selected for. We set out therefore to investigate whether there is any association between the protective immune response against *falciparum* infections and FcγRIIa and FcγRIIIb allotypes.

1.1.2 Objectives

The aims of the present study are to find out if (i) there are any differences in the distribution of Fcγ receptors among Ghanaians and (ii) to determine if there is any association between the FcγRIIa and FcγRIIIb and protective immune response against *falciparum* malaria. These will be achieved by conducting a case study on a number of children diagnosed as having uncomplicated malaria, cerebral malaria or severe anaemia.

The specific objectives were as follows

(i).To recruit and obtain demographic data on age, sex and ethnicity of a cohort of children.

(ii). To categorize the children according to their disease status.

(iii). To determine their respective FcγRIIa and FcγRIIIb genotypes by PCR.

When the data on the above were obtained, they were then analysed further to:

(i) determine the distribution of the FcγRIIa and FcγRIIIb genotypes within the different ethnic groups,

(ii) determine if there is any association between the FcγRIIa and FcγRIIIb genotypes and ethnic origin,

(iii) find out if there is any association between FcγRIIa and FcγRIIIb and the disease status of the patients,

(iv) compare the genotype frequencies of the patients with those of the controls.

1.2 LITERATURE REVIEW

1.2.1 Immnoglobulins

Immunoglobulins (Ig), also called antibodies are a group of serum molecules produced by B lymphocytes. Immunoglobulin proteins are the critical ingredients at every stage of a humoral immune response. When expressed on the surface of resting B-lymphocytes, they serve as receptors that can detect and distinguish among the vast array of potential antigens present in the environment (Benjamini, 1996). The two hallmarks of immunoglobulins as antigen binding proteins are the specificity of each for a particular antigenic structure and their diversity as a group. In addition to antigen binding, immunoglobulins possess secondary biologic activities that are critical for host defence (Goodman and Parslow, 1994).

Based upon the structure of their heavy chain constant region, there are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM with their heavy chains designated as α, δ, ε, γ and μ, respectively. All antibodies have basically the same structure, but they are diverse in the region that binds to the antigen. They also differ in amino acid composition, making them different in size and charge (Roitt, 1988).

In addition to differences between classes, the immunoglobulins within each class are also very heterogeneous. For example, there are four subclasses of human IgG (namely IgG1, IgG2, IgG3 and IgG4). There are also known to be subclasses of human IgA (IgA1 and IgA2), but none have been described for IgD, IgM and IgE (Silverton *et al.*, 1977).

Each immunoglobulin is bifunctional (Parslow, 1994). One region of the molecule is concerned with binding to antigen while a different region mediates so-called

effector functions. These include binding of the immunoglobulin to host tissues, including various cells of the immune system, some phagocytic cells, and the first component (C1q) of the classical complement system (Kuby, 1992).

All immunoglobulins are glycoproteins with the carbohydrate content ranging from 2-3% to 12-14% (Roitt, 1988). The antibody molecule is made up of two identical heavy and light polypeptide chains held together by disulphide bonds. These chains can be separated by reduction of the S-S bonds and acidification. The exposed hinge region is extended in structure due to the high proline content and is therefore susceptible to proteolytic attack; thus the molecule can be digested by papain to yield two identical fragments, each with a single combining site for antigen (Fab; *fragment antigen binding*), and a third fragment which lacks the ability to bind antigen and is termed the Fc (*fragment crystallisable*). Pepsin acts at a different point to cleave the Fc portion from the divalent F $(ab)_2$ (Valentine & Green, 1967). Of all the antibodies, IgG is the major immunoglobulin in normal human serum, accounting for 70-75% of the total immunoglobulin pool (Roitt, 1988).

1.2.1.1 Immunoglobulin G

IgG is the predominant isotype present in blood and extracellular fluid and is primarily responsible for providing systemic immune protection. It consists of a single immunoglobulin molecule with a sedimentation coefficient of 7S and a molecular weight of 146 kDa (Burton & Woof, 1992). The IgG class that is distributed evenly between the intravascular and extravascular pools is the major antibody of secondary immune responses and the exclusive antitoxin class. Through its ability to cross the placenta, IgG provides a major line of defence against infection for the first few weeks of a baby's life which may be further reinforced by the

transfer of colostral IgG across the gut mucosa in the neonate through breast-feeding (Silverton, 1977).

1.2.2 Fc Receptors

Many cell types that cannot synthesize immunoglobulins are able to adsorb circulating antibodies by way of the Fc receptors (Alazari *et al.*, 1988). These receptors, found on the surface of leucocytes, are so called because they interact with the Fc portion of the heavy chain of immunoglobulins (Davis and Metzger, 1983).

The physiologic functions of Fc receptors differ among cell types. Fc receptors facilitate phagocytosis of antibody-coated particles through the phenomenon of opsonization, and are important for triggering chemotaxis and degranulation in neutrophils and other phagocytes. Binding of IgG molecules onto these Fc receptors on macrophages or natural killer cells serves to arm these cells to carry out Antibody dependent cellular cytitoxicity (ADCC) (Goodman and Parslow, 1994).

1.2.2.1 Receptors for Immunoglobulin G (IgG)

Receptors for IgG (FcγR), a subgroup within the larger group of FcR, were shown to belong to the Ig supergene family (Van de Winkel & Anderson, 1991). Three major classes of leukocyte FcγR are currently recognized, all of which contain several members.

Within an individual, Fcγ receptor diversity may be related to differences in primary amino acid structure, variation between specific cell types or the association of different molecules during receptor assembly (Kimberly *et al*, 1995). In humans there are eight distinct Fcγ receptor genes, with multiple exons, with the result that

alternative splicing can generate distinct protein products. All three Fcγ receptor families share similar though non-identical extracellular (EC) domains, comprising a common structural motif of two or three immunoglobulin-like disulphide linked domains, dictating their ligand- binding characteristics (Ravetch & Anderson, 1990).

1.2.2.2 General characteristics of human FcγR

Most FcγR exist as hetero-oligomeric complexes and as such, belong to the family of multichain immune recognition receptors. The ligand binding-α-chains can associate with a number of signalling components. The three FcR signalling chains and the α-chain of FcγRIIa bear a unique 26-amino acid immuno-receptor tyrosine-based activation motif in their cytoplasmic domain (Rascu *et al.*, 1997).

FcγRI (CD64) is a 72-kDa glycoprotein with high affinity receptor for IgG. The receptor is heavily glycosylated and has a core protein, revealed after removal of N-linked carbohydrates with a MW of 55kDa (Peltz *et al.*, 1988). This group of receptors are constitutively expressed on monocytes, macrophages, myeloid progenitor cells, dendritic cells, and are also inducible on neutrophils by interferon-γ (IFN-γ) or granulocyte colony-stimulating factor (Guyre *et al.*, 1983; Repp *et al.*,1991).

Three homologous genes, FcγRIA, FcγRIB and FcγRIC, encode members of the Class-I FcγR and are localized on chromosome 1. FcγRIa and FcγRIb2 are transmembrane molecules with their extracellular regions composed of three (FcγRIa) or two (FcγRIb2) Ig-like domains, respectively. The human (h)FcγRI gene is composed of six exons, two exons encoding the signal peptide, one exon for each

of the Ig-like domains, and a single transmembrane /cytoplasmic region exon (Van de Winkel *et al.,* 1990).

Human (h) FcγRI is the only receptor class that binds monomeric Ig with high affinity. It shows specificity for three subclasses, hIgG1, hIgG3 and hIgG4 in order of decreasing affinity, and interacts with mouse (m) IgG2a and mIgG3, as well as rat IgG2b antibodies (Anderson & Abraham, 1980).

A family of 40-kDa FcγRII (CD32) molecules represent the most broadly distributed Fcγ receptor class. FcγRII members are expressed on most types of blood leukocytes, Langerhans' cells, different population of endothelial cells, dendritic cells, macrophages and platelets. Three genes, FcγRIIA, FcγRIIB, and FcγRIIC, located on chromosome 1 encode six isoforms (FcγRIIa1, IIa2, IIb1, IIb2, IIb3 and IIc), which differ profoundly in their cytoplasmic tails (Qiu *et al.*, 1990). The hFcγRIIA and B gene products differ in both their signal peptides and cytoplasmic domains. The hFcγRIIC gene contains a cytoplasmic region similar to h FcγRIIA, and a signal peptide that is closely related to that of the hFcγRIIB gene (Brooks *et al.,* 1989). Analysis of the amino acid sequences from cDNAs of the multiple isolated receptor isoforms indicates that the proteins of this receptor class have a similar extracellular region of about 180 amino acids that contain two Ig-like domains, a 27 to 29 amino acid transmembrane domain and a cytoplasmic domain that varies in length from 44 to 76 amino acids (; Hibbs *et al.*, 1988; Brooks *et al.*, 1989 and Seki, 1989). FcγRII is a low affinity receptor, interacting only with complexed or aggregated IgG and is the sole FcγR class that can bind hIgG2 (Kimberly, 1995).

FcγRIII (CD16) is a glycoprotein with a molecular weight ranging from 50 to 80 kDa. Two different genes have been identified, FcγRIIIA and B, located on chromosome 1 within 200 kb from the FcγRII genes (Van de Winkel & Anderson, 1991). The products of both genes encode proteins with an extracellular region of 190 amino acids containing Ig-like domains. The most remarkable difference between the two subclasses is that hFcγRIIIa is a transmembrane receptor (with a 25 amino acid cytoplasmic region) and hFcγRIIIb is coupled to the outer leaflet of the plasma membrane via a glycosylphosphatidylinositol (GPI) moiety (Huizinga *et al.*, 1988; Selvaraj *et al.*, 1989). The two genes are highly homologous and differ most critically at amino acid position 203, where a serine in hFcγRIIIb determines the GPI-linked molecule, whereas a phenylalanine in hFcγRIIIb specifies preservation of the transmembrane and cytoplasmic domains to generate an integral membrane glycoprotein (Kurosaki & Ravtech, 1989; Lanier *et al.*, 1989).

FcγRIIIa is present on (NK) cells, macrophages, subpopulations of T cells and freshly isolated blood monocytes, immature thymocytes, and placental trophoblasts and binds IgG with medium affinity. FcγRIIIb is selectively expressed on neutrophils and is a low affinity receptor for IgG. Both isoforms effectively bind hIgG1 and hIgG3 and interact with mouse IgG (Rascu *et al.*, 1997).

Besides membrane bound forms of FcγR, much evidence also suggests the existence of soluble receptors. Huizinga *et al.* (1990), have shown that normal human sera contain a high amount of soluble hFcγRIII receptor, most likely from neutrophils. This receptor seems to be released from PMN by a proteolytic mechanism. Another

way of generating these soluble receptors might be through alternative splicing. Warmerdam *et al.* (1990), have described a hFcγRII form that lacks the transmembrane region and presumably encodes a soluble molecule.

1.2.2.3 Polymorphisms of FcγR

Another form of variation in the structure of immunoglobulins is allotypy. It is based on genetic differences between individuals and depends on the existence of allelic forms (allotypes) of the same protein as a result of the presence of a different amino acid of the same gene at a given locus. As a result of allotypy, a particular constituent of any immunoglobulin can be present in some members of a species and absent in others (Alazari *et al.,* 1988). This situation contrasts with that of immunoglobulin classes which are present in all members of a species.

Allotypic differences at known loci usually involve changes in only one or two amino acids in the constant region of a chain. With a few exceptions, the presence of allotypic differences in two identical immunoglobulin molecules does not generally affect binding with antigen but it serves as an important marker for analysis of Mendelian inheritance (Goodman and Parslow, 1994).

FcγRIa is not polymorphic, although a single family has been identified in Belgium, lacking phagocyte expression of FcγRIa (Ceuppens *et al.,* 1988). This absence was recently linked to a single nucleotide difference between the wild type FcγRIa and the Belgium family members lacking this receptor. Interestingly, in spite of the absence of FcγRIa, the four members of this family were apparently healthy, lacking evidence for any autoimmune, infectious, or inflammatory disease.

A well-studied polymorphism represents allelic variation within the myeloid FcγRIIa (CD32). A point mutation, G into A, results in an arginine (R131) or histidine (H131) at amino acid position 131 in the second Ig–like domain of FcγRIIa (Warmerdam *et al.,* 1990). This variation has been shown to be critical for the binding of hIgG2 and hIgG3, as well as mIgG1 and rat IgG2b (Warmerdam *et al.,* 1991; Parren *et al.,* 1992). An independent polymorphic amino acid is located at position 27 (either glutamine or tryptophan) which is not known to be relevant to the ligand binding of FcγR (Warmerdam, 1990).

This genetically determined polymorphism of hFcγRII was originally discovered in studies on the interaction between human monocytes and mIgG1 antibodies stemming from analysis of anti-CD3-induced T-cell proliferation. Monocytes from 70% of Caucasian individuals (high responders, HR) supported the T-cell mitogenesis induced by mIgG1 anti-CD3 mAb, whereas 30% did not (low responders, LR) (Tax *et al.,* 1983). These findings were confirmed in other functional assays such as EA-rosetting, phagocytosis and ADCC of mIgG1-opsonized targets, binding of mIgG1 aggregates, mIgG1-induced release of tumour necrosis factor-α (TNF-α), and adherence of PMN to mIgG1-coated endothelial cells (Van de Winkel & Anderson, 1991).

The relative predominance of hFcγRIIa transcripts in three cell types, monocytes, macrophages, and neutrophils, that express this polymorphism suggests that, of the three FcγRII genes, only hFcγRIIA bears the allotypic polymorphism. The observation that B cells from all donors uniformly bind mAb 41H16 and express

only hFcγRIIb supports this hypothesis (Gosselin *et al.*, 1990). Information about the *in vivo* relevance of this polymorphism is scant. The FcγRIIb subclass has also been shown to exhibit genetic heterogeneity based on a single amino acid difference at position 11 in the cytoplasmic region, where a tyrosine is substituted by an aspartic acid (Warmerdam *et al.*, 1993).

A second type of heterogeneity of FcγRII has been described on human platelets. A stable variation in expression of numbers of hFcγRII on platelets from different individuals was found, which correlated with intensity of the platelet release reaction and platelet aggregation in response to model immune complexes. These workers speculate that this variation in quantitative expression may be associated with susceptibility to certain immune complex disease (Rosenfeld *et al.*, 1987).

The FcγRIIa-H131 allotype has been shown to effectively bind hIgG2 dimers, hIgG2 anti-CD3 antibodies and hIgG2-opsonized bacteria, in contrast to the FcγRIIa-R131-expressing cells (Salmon *et al.*, 1992). Similarly, rat IgG2b-opsonized red blood cells and rat IgG2b anti-CD3 mAb were much more effectively bound by FcγRIIa-H131-expressing cells.

The third FcγR class bears a structural polymorphism known as the NA system. Represented only on hFcγRIIIb expressed by neutrophils, it is involved in autoimmune diseases and transfusion reactions. This polymorphism is reflected in different molecular weights of the deglycosylated FcγR on SDS-PAGE. Human (h) FcγRIIIb from NA1/NA1 donors has a MW of 29kDa; that of NA2/NA2 donors has an MW of 33kDa, and heterozygous donors express both. The sequences of the

cDNA of these allotypic forms reveal minor amino acids differences between the NA1 and NA2 forms of hFcγRIIIb (Ory *et al.,* 1989; Ravtech & Perussia, 1989). Changes in amino acids at positions 63 and 82 lead to two extra glycosylation sites (six instead of four) in the NA2 form resulting in different electrophoretic mobilities of the two allotypes (Huizinga *et al.,* 1990; Ory *et al.,* 1989). PMN from FcγRIIIb-NA2 individuals were consistently found to exhibit lower levels of phagocytosis of erythrocytes either sensitised with hIgG1 and IgG3 anti-Rhesus D mAb or treated with concanavalin A compared to FcγRIIIb-NA1-PMN (Salmon *et al.,*1990; Bredius *et al.,*1994). Furthermore, phagocytosis of hIgG1-opsonized bacteria by FcγRIIIb-NA2-PMN was also reduced in comparison to FcγRIIIb-NA1-PMN, whereas no difference was found using hIgG2-opsonized bacteria (Bredius *et al.,* 1994).

Recently a triallelic polymorphism of FcγRIIIa has been identified, based on a single nucleotide difference (position 230), leading to a leucine, histidine, or arginine at position 48 in the first IgG—binding characteristics between the three FcγRIIIa allotypes (De Haas *et al.,* 1996).

Another polymorphism of FcγRIII was described by Ravtech and Perussia (1989), where a nucleotide substitution at position 559 of FcγRIIIa predicts either a valine or a phenylalanine at amino acid position 158 of protein product. The third type of interindividual variation in FcγRIIIa represents an expression difference reported by Vance *et al.* (1993). Individuals were identified with either a high or low level of CD 16 expression on NK cells, supportive of a polymorphism. Functional differences were furthermore noted in NK-cell ADCC activity and binding of

monomeric hIgG. However a genetic basis for this heterogeneity remains to be established.

Two groups have independently identified individuals who lacked expression of hFcγRIIIb. Clark *et al* .(1990), identified a patient with systemic lupus erythematosus (SLE) with no FcγRIII on her PMN. Huizinga *et al.* (1990), studied two healthy individuals both of whom lacked hFcγRIIIb. In both studies the defect seemed attributable to either a grossly disorganized, or a completely absent gene. In the latter study the two deficient individuals were healthy and had no signs of circulating immune complexes or increased susceptibility to infections. These observations raise questions about the *in vivo* relevance of this receptor class and support the notion that the Fcγ receptor class are structurally redundant.

1.2.2.4 Ethnic Variation in Frequency of Allelic Polymorphism of Human FcγR

The frequency of these allotypic polymorphisms varies among ethnic groups. The percentage of individuals with cells responsive to mIgG1 in the T cell mitogenic assay differs greatly among genetically diverse populations (Abo *et al.,* 1984). East Asians (Japanese, Chinese, Indians) are more frequently of the mIgG1 low responder phenotype as compared to a caucasian group. Such variation may, in part, explain racial differences in the susceptibility to certain diseases such as those caused by *Haemophilus influenzae* and *Neisseria* (Nagata *et al.,* 1989; Musser *et al.,* 1990; Figueroa & Densen, 1991).

Osborne *et al.* (1994), showed that the frequency in these ethnic groups of the high and low responder mIgG1 phenotype correlates directly with the R/H131 polymorphism and therefore with the capacity of FcγRII to bind hIgG2 with high

affinity. In healthy Caucasians, the RIIIb-NA2/NA2 phenotype is expressed more often (Lalezari, 1984; Sanders *et al.*, 1994), whereas the RIIIb-NA1/NA1 predominates in Japanese individuals (Ohto & Matsuo, 1989) (Table 1.1). The NA-null gene was assessed to be 0.03 in a French population.

Table 1.1 Phenotypic Distribution of FcγRIIa and FcγRIIIb Allotypes in Different Ethnic Populations

Ethnic Groups	FcγRIIa(%)				FcγRIIIb(%)				References
	N	R/R	R/H	H/H	N	NA1/NA1	NA1/NA2	NA2/NA2	
Caucasian									
Dutch	123	23	48	29					Sanders *et al.*, 1994
German	187	27	45	28					"
American	35	23	54	23	67	15	45	40	Osborne *et al.*, 1994 & Wainstein *et al.*, 1995
French					3377	12	43	45	Fromont *et al.*, 1992
Asians									
Japanese	27	6	33	61	303	42	39	9	Osborne *et al.*, 1994 & Ohto & Matsuo, 1989
Chinese	18	6	44	50					Osborne *et al.*, 1994
Indian	16	31	56	13					"
African-American	100	23	50	27					Salmon *et al.*, 1996

N = Number of individuals assessed

1.2.2.5.Clinical Relevance of FcγR Polymorphism

FcγRs are of critical importance in directing the uptake and destruction of viruses, bacteria and a variety of infectious parasites, and are involved in antibody-dependent killing of infected cells expressing viral antigens (Wallace *et al.,* 1995; Van de Winkel and Capel, 1996). FcγRIIa-expressing NK cells isolated from human immunodeficiency virus (HIV)-seropositive individuals have been shown to be coated with anti-HIV antibodies and readily mediate lysis of HIV-infected cells *in vitro*. Furthermore, this ADCC activity correlates inversely with disease progression. The importance of appropriate detection of IgG-opsonized microorganisms by FcγRs on phagocytes is further emphasized by susceptibility for individuals expressing the FcγRIIa-R131 allotype to infections by encapsulated bacteria (Tse *et al.,* 1999).

The FcγRIIa-H131 allotype (as opposed to FcγRIIa-R131) is identified as the only FcγR capable of binding human IgG2, an important isotype in immune defence against encapsulated bacteria (Parren *et al.*, 1992; Bredius *et al.,* 1993). Neutrophils from individuals expressing the FcγRIIa-R131 allotype inefficiently phagocytose human IgG2-coated bacteria, rendering these individuals more susceptible to infection. Allotypic forms of FcγRIIIb (NA1 versus NA2) have also demonstrated differences in the binding and phagocytosis of IgG1- and IgG3-coated particles, which may have clinical relevance with regard to susceptibility to infectious disease. (Salmon *et al.,* 1992).

FcγRs are also important for immune defense to intracellular pathogens such as *Toxoplasma gondii* (Deo *et al.,* 1997). Antibodies specific for *T. gondii* focus the organism to the effector cell by binding to FcγRs, thereby leading to destruction of

the pathogen. Bispecific molecules (BSMs) that focus *T. gondii* to the surface of myeloid effectors (monocytes and neutrophils) mediate destruction of the pathogen regardless of the surface antigen on the effector cell to which they are directed (Erbe *et al.,* 1991). In contrast to phagocytes, NK cells destroy *T. gondii* only upon targeting to FcγRIII, and not other cell-surface markers, identifying FcγRIII on NK cells as primary trigger molecule for *T. gondii* destruction (Erbe *et al.,* 1991). BSMs are now being developed for a variety of microorganisms, including fungi and antibiotic-resistant bacterial strains, to target these pathogens specifically to FcγR-expressing cytotoxic effector cells (Deo *et al.,* 1997).

Porges *et al.* (1994), demonstrated murine IgG anti-neutrophil cytoplasmic antibodies (ANCA), which are associated with Wegener's granulomatosis and systemic vasculitis, to engage and activate human neutrophils via FcγRIIa. The production of reactive oxygen intermediates after incubation with mIgG1 ANCA was significantly increased in individuals expressing the IIa-R/R131 phenotype. These data are in accordance with those of Mulder *et al.,* (1994) who concluded that ANCA –induced activation of neutrophils was FcγRIIa-dependent, and correlated with hIgG3 ANCA titres in patients with Wegener's granulomatosis. However, this increased neutrophil activation in FcγRIIa-R/R131 donors almost certainly reflects the greater avidity of these receptors for murine IgG1 (Clark *et al.,* 1991; Tate *et al.,* 1992; Porges *et al.,* 1994). The situation with human IgG was unclear.

FcγRIIa receptor allotypes may represent risk factors in ANCA-associated systemic vasculitis, and influence susceptibility or disease manifestations if IgG2 and / or IgG3 are the predominant isotype. Since the FcγRIIa-H/H131 receptor binds human

IgG3 with greater avidity and also binds IgG2, receptor engagement and antigen recognition by ANCA could lead to an enhanced neutrophil activation and tissue injury, compared to the FcγRIIa-R/R131 receptor. Thus allelic variants of neutrophil FcγR may contribute to disease susceptibility and organ involvement through differential activation by ANCA isotypes (Tse *et al.,* 1999). Tse *et al.* (1999) however found that there was no significant increase of the FcγRIIa-H/H131 allotype amongst patients with ANCA-positive systemic vasculitis, irrespective of ANCA specificity, and also that there was no association between this FcγRIIa allotype and nephritis.

Depending on the ANCA IgG subclass specificity, FcγRIIa alleles may thus represent heritable disease risk factors in ANCA-triggered inflammation in patients with Wegener's granulomatosis and systemic vasculitis.

Heparin-induced thrombocytopenia (HIT) is characterized by FcγR-mediated platelet activation in the presence of patient serum and heparin and is diagnosed by evaluating the effect of patient plasma on the aggregation of normal donor platelets. Brandt *et al.* (1995), showed that platelets from donors bearing the RIIa-H/H131 phenotype were largely unresponsive to plasma from patients with HIT or to murine IgG1 anti-platelet mAb. The frequency of the RIIa-H/H131 allotype was significantly increased in patients with HIT (34.4%) compared to nonthrombocytopenic patients (19%), suggesting a pathophysiologic role of the FcγRIIa polymorphism in HIT (Brandt *et al.,* 1995).

FcγRs have been shown to play a significant role in autoimmune disorders, either by meditating destruction of normal cells opsonized with autoantibodies or conversely,

by failing to clear immune complexes (ICs) adequately. For example, inability of FcγR-bearing cells to remove soluble ICs has been proposed to enhance autoimmune conditions such as systemic lupus erythematosus (SLE), where IC deposition in tissues triggers inflammation and tissue destruction, a characteristic type III hypersensitivity reaction (Deo *et al.,* 1997). On the other hand, engagement of functional FcRs on effector cells of the mononuclear phagocyte system triggers the destruction of autologous erythrocytes or platelets in the presence of autoantibodies directed to these cells. This results in autoimmune haemolytic anaemia or idiopathic thrombocytopenia purpura, both of which are autoimmune disorders characteristic of type II hypersensitivity class of inflammation (Deo *et al.*, 1997).

Two SLE patients were identified lacking FcγRIIIb expression, based on either a transport defect of one of the receptor alleles (Enenkel *et al.,* 1991) or an absent / abnormal gene (Clark *et al.,* 1990). It was tempting to speculate that the FcγR polymorphism may underlie the FcγR- mediated phagocytosis described in SLE patients. Indeed, Blasini *et al.* (1993), described an increased proportion (82%) of T cell responders to Leu4 (mIgG1 anti-CD3 mAb), expressing the RIIa-R/R131 or RIIa-R/H131 allotypes, in Venezuelan SLE patients, compared to patients with other autoimmune diseases. Duits *et al.* (1995) found a significant association between the RIIa-R/R131 allotype and lupus nephritis (38% for RIIa-R/R131, 48% for RIIa-R/H131, and 14% for RIIa-H/H131) as compared to either SLE without nephritis (18% RIIa-R/R131, 58% RIIa-R/H131 and 24% RIIa-H/H) or healthy Caucasian donors. No differences were found for FcγRIIIb polymorphism between SLE patients with or without nephritis.

In line with some studies made by Duits *et al.* (1995), Salmon *et al.* (1996), also reported a skewed distribution of the RIIa-H/H131 genotype (9%) in African American SLE patients compared to normal donors (30%). These differences were even more striking in a large collection of 214 SLE patients, where 37% expressed the RIIa-R/R131 allotype compared to 23% RIIa-R/R131 homozygotes in normal donors. The highest frequency of the RIIa-R/R131 allotype was observed in SLE patients with nephritis (42% RIIa-R/R131 and 12% RIIa-H/H131).

Due to the similarities in the pathogenesis of SLE and malaria, it may be possible that FcγR polymorphisms are likely to play a major role in an individual's susceptibility to malaria.

1.2.3 Malaria: The Disease

Malaria is an infectious disease caused by protozoa of the genus *Plasmodium* which, in sub-Saharan Africa is transmitted by *Anopheles gambiae* mosquitoes. Malaria is a major global health problem posing a threat to up to 40% of the world's population (WHO, 1996). The WHO reports that malaria in sub-Saharan Africa has an intense stable transmission causing 270-480 million clinical cases each year and affecting mainly young children.

There are over 120 species of *Plasmodia,* but only four are responsible for malaria in humans with each species causing characteristic symptoms. The four species are *Plasmodium falciparum, P. malariae, P. ovale* and *P. vivax* and each of these is biologically and morphologically distinct.

In Africa, *P. falciparum* predominates, as it does also in Papua New Guinea and Haiti, and it is responsible for all lethal forms of malaria, whereas *P. vivax* is more common in Central and parts of South America, North Africa, the Middle East and the Indian subcontinent. The prevalence of both species is approximately equal in other parts of South America, East Asia and Oceania. *Plasmodium vivax* is rare in sub-Saharan Africa, whereas *P. ovale* is rare outside West Africa . *Plasmodium. malariae* is found in most areas , but is relatively uncommon outside Africa (White, 1996).

The endemicity of malaria is defined traditionally in terms of the spleen or parasite rates in children aged between 2 and 9 years (Garnham, 1966). In areas which are holoendemic or hyperendemic for *P. falciparum,* such as much of tropical Africa, people are repeatedly infected throughout their lives (Cattani *et al.,* 1986). There is considerable morbidity and mortality during childhood. In the Gambia, where people are infected once each year on average, malaria has been estimated to cause 25% of deaths between 1 and 4 years of age, but eventually if the child survives, a state of premunition is achieved where infections cause little or no problems to the host (White, 1996). Non-immune adults entering an area of intense transmission acquire premunition more rapidly than children.

Babies develop severe malaria relatively infrequently (although if they do, mortality is high). The factors responsible for this include passive transfer of maternal immunity (McGregor, 1984), and the high haemoglobin F content of the infants' erythrocytes which retards parasite growth (Pasvol *et al.,* 1977). In holoendemic areas, the baby is inoculated repeatedly with sporozoites during the first year of life, but the blood stage infection is seldom severe.

The pathophysiology of malaria results from destruction of erythrocytes, the liberation of parasite and erythrocyte material into the circulation, and the host's reaction to these events. *Plasmodium falciparum* malaria-infected erythrocytes also sequester in the microcirculation of vital organs, interfering with microcirculatory flow and host tissue metabolism (Ponnudurai *et al.*, 1991).

Malaria can be uncomplicated or severe. Uncomplicated malaria is defined as malaria in the absence of the features of severe malaria (WHO, 1990). It is the most common presentation of malaria. Clinical features in children include fever, headache, anaemia, vomiting and watery diarrhoea, tachypnoea, cough and febrile convulsions. Uncomplicated malaria is not immediately life threatening, but must be treated promptly because it can rapidly lead to severe anaemia.

Severe malaria is defined as asexual with one or more of the following complications; coma (cerebral malaria), respiratory distress, hypoglycaemia and severe anaemia. These are the most common causes of death due to malaria in children. There can also be repeated generalized convulsions, metabolic acidosis and shock. Other features of severe malaria in children are impaired consciousness other than coma, prostration or extreme weakness, hyperparasitaemia, jaundice and a rectal temperature above 40°C (White, 1996). In adults, additional defining features of severe malaria are acute renal failure, pulmonary oedema including adult respiratory distress syndrome (ARDS), spontaneous bleeding and haemoglobinuria (black water fever). These features are less common in children (WHO, 1990).

1.2.3.1 Cerebral Malaria

This may be defined as unrousable coma, i.e. there is a non-purposeful or no response to a painful stimulus in *falciparum* malaria (Molyneux *et al.*, 1989). Although cerebral malaria is the most predominant feature of severe *falciparum* malaria, some patients with ultimately lethal infections never lose consciousness until they die. The onset of coma may be sudden, often following a generalized seizure, or gradual, with initial drowsiness, confusion, disorientation, delirium or agitation, followed by unconsciousness. The length of the prodromal history is usually several days in adults, but in children can be as short as 6-12 hours. The depth of coma is classified by a modification of the Blantyre coma scale (Table 1.2) (WHO, 1990).

Table 1.2. Blantyre coma scale for assessment of consciousness in young children

Best Motor Response	**Score**
Localizes painful stimulus	2
Withdraws limb from painful stimulus	1
No response	0
Best Verbal Response	
Cries or speaks appropriately with painful stimulus	2
Moan or abnormal cry with painful stimulus	1
No vocal response to painful stimulus	0
Eye Movement	
Watches or follows	1
Fails to watch or follow	0
Total Score	0-5

Untreated cerebral malaria is probably uniformly fatal. The overall mortality of treated cerebral malaria obviously depends on the referral practices and medical facilities available, but in reported studies averages 15% in children and 20% in adults, but up to 50% in pregnancy (White & Ho, 1992).

The occurrence of cerebral malaria appears to have a direct relationship to parasite levels in blood, with better nourished children more severely affected (Hendrickse *et al.,* 1971). Cerebral malaria is not uncommon in Accra, Ghana. Mortality associated with cerebral malaria has been claimed to be as high as 10-20% even with the best of care (Nkrumah, 1977; Commey *et al.*, 1980). Over the past decade, workers in

Accra, Ghana have noted and reported a considerable increase in the incidence of cerebral malaria, especially among older children (Commey *et al.,* 1980).

Presumably the metabolic milieu created adjacent to the sequestered and highly metabolically active parasites interferes with neurotransmission but how this occurs is not known. Cytokines increase production of nitric oxide, a potent inhibitor of neurotransmission, by leucocytes, smooth muscle cells, microglia and vascular endothelium through induction of the enzyme nitric oxide synthase. Local synthesis of nitric oxide could well be relevant to the impairment of consciousness. Coma in malaria is not caused by raised intracranial pressure (White, 1996).

1.2.3.2 Anaemia in malaria

The degree of anaemia and the rate at which it develops vary enormously. The haemoglobin concentration may fall by up to 2 g/dl each day (Mabey *et al.,* 1987). Some patients appear to tolerate severe malarial anaemia relatively well. These patients usually have an underlying chronic anaemia, and have adapted to increased oxygen carriage. Thus it is both the absolute haemoglobin concentration and the magnitude of the fall that determine the clinical consequences (Lee *et al.,* 1989).

The pathogenesis of anaemia is multifactorial (Zuckerman, 1966; Perrin *et al.*, 1982). There is obligatory destruction of red cells containing parasites at merogony. There is also accelerated destruction of non-parasitized cells that parallels disease severity (Davis *et al.,* 1990; Looareesuwan *et al.*, 1991). In severe malaria, anaemia develops rapidly; as there is also the rapid haemolysis of unparasitized red cells contributing to the decline in haematocrit (Looareesuwan *et al.,* 1987).

The role of antibody in anaemia is unresolved (Facer *et al.,* 1979; Abdallah *et al.,* 1980; Merry *et al.,* 1986). The majority of studies to date do not show increased red cell immunoglobulin binding in malaria, but in the presence of a lowered recognition threshold for splenic clearance, this might be difficult to detect. Red cell survival is shortened in malaria and this is affected by corticosteroids (Charoenlarp *et al.,* 1979).

1.2.4 Immune Response

The precise mechanisms controlling malaria infections are still incompletely understood. It was apparent from the era of malaria therapy, that a strain-specific immunity developed to protect against re-challenge with the same strain, did not protect from infection with a different strain (Jeffery, 1966).

Immunity, as distinct from premunition, may be reached when there has been exposure to all local strains of malaria parasites. In controlling acute infection, non-specific host defence mechanisms and the development of more specific cell-mediated and humoral responses are both important.

Acute malaria infections are associated with malaria antigen-specific unresponsiveness. This selective paresis is one of the factors contributing to the slow development of an effective and specific immune response to malaria. In non-immune individuals, the acute immune response to infection often comprises mostly IgM and IgG2 isotypes which are unable to harm cytotoxic cells and thus kill asexual malaria parasites (Bouharoun-Tayoun & Druilhe, 1992). These observations have led to the suggestion that malaria induces an immunological 'smokescreen' with broad-spectrum and non-specific activation that interferes with the orderly development of a specific cellular immune response (Ho & Webster, 1990).

Following an infection, there is a transient humoral response to sporozoite antigens; sporozoites antibodies decline, with a half-life of 3-4 weeks (Webster *et al.,* 1988). In areas of high transmission, sporozoite antibody levels tend to plateau between 20 and 30 years of age, and do not correlate with premunition.

Protective antibodies inhibit parasite expansion through co-operation with the monocyte-macrophage series by binding to parasitized erythrocytes and then activating these cells' Fc receptors (Bouharoun-Tayoun *et al.,* 1990). Non-specific effector mechanisms include the activation of phagocytic cells (including neutrophils) to release toxic oxygen species and nitric oxide, both of which are parasiticidal. The reaction of these oxygen intermediates with lipoproteins produces lipid peroxides (Rockett *et al.,* 1988). These are more stable cytotoxic molecules and are unaffected by antioxidants. There is also augmentation of splenic clearance function in which both filtration (Looareesuwan *et al.,* 1987), and Fc receptor-mediated phagocytosis are increased (Lee *et al.,* 1989; Ho *et al.,* 1990).

Infected erythrocytes are more rigid and more opsonized than uninfected red cells that express both host and parasite-derived neoantigens on the erythrocyte surface. However, the parasite proteins expressed on the red cell surface undergo antigenic variation (Marsh & Howard, 1986; Hommel & Semoff, 1988), and this is probably instrumental in avoiding complete immune clearance and sustaining the infection. The monocyte-macrophage series appear to be the most immune effector cells in the direct attack on parasitized erythrocytes and merozoites, although neutrophils may also play a part.

IgG diffuses more readily than the other immunoglobulins into the extravascular body spaces where, as the predominant species, it carries the major burden of neutralizing bacterial toxins and binding to micro-organisms to enhance their phagocytosis (Benjamini, 1996). The complexes of bacteria with IgG antibody activate complement thereby chemotactically attracting polymorphonuclear phagocytic cells (PMNs), which adhere to the bacteria through surface receptors for complement and the Fc portion of the IgG (Fcγ). Binding to the Fc receptor then stimulates ingestion of micro-organisms through phagocytosis. In a similar way, the extracellular killing of target cells coated with IgG antibody is mediated largely through recognition of the surface Fcγ by NK cells bearing the appropriate receptors (Cresswell, 1987).

The interaction of IgG complexes with platelet Fc receptors presumably leads to aggregation and vaso active amine release, but the physiological significance of Fcγ binding sites on other cell types, particularly lymphocytes, has not yet been clarified (Roitt, 1988).

1.2.5 Methods for FcγR Genotyping and Phenotyping

1.2.5.1 Immunological Methods

Neutrophil-specific antigens, NA1 and NA2 can be typed by leucoagglutination with a panel of anti-NA1and anti-NA2 allosera (Huizinga *et al.,* 1990). Whereas the cross linking of multivalent protein antigens by antibodies leads to precipitation, cross-linking of cells or large particles by antibodies directed against surface antigens lead to agglutination.

Neutrophil-specific antigens NA1 and NA2 can also be determined serologically by radioimmunoassay (RIA) with Murine Antibodies CLB-FcR gran I, CLB-gran II and GRMI (Edberg *et al.,* 1990). Using sera from donors known to be FcγRIIIb-NA1/NA1 positive, the binding of CLB gran II relative to a standard is determined and set as 100% relative binding. Phenotyping can also be done by flow cytometry using CD16 MAb ID3, which selectively reacts with FcγRIII (Perussia and Ravtech, 1991), MAb CLB gran II which specifically recognizes FcγRIIIb-NA1 (Werner *et al.*, 1986; Huizinga *et al.,* 1989), and MAb GRMI, which detects FcγRIIIb-NA2 (Edberg *et al.,* 1990).

Determination of the H131 / R131 alleles can be done by quatitative flow cytometry using CD32 MAb IV.3, which reacts with both FcγRIIa-H131 and –R131 (Looney *et al.,* 1986) and MAb 41H 16, which reacts selectively with FcγRIIa-R131 (Gosselin *et al.*, 1990)

The FcγRIIa phenotype of cells from different donors can be analysed by comparing the potency of mIgG1 and hIgG2 blood. Three groups of donors can be discriminated: two groups with PBMC only responsive to either mIgG1 or hIgG2 anti-CD3MAb, and one group with a comparable reactivity to both anti-CD3 MAb, indicating homozygous FcγRIIa-R131, homozygous anti-CD3-MAb induced Tcell proliferation in PBMC isolated from citrated FcγRIIa-H131, and heterozygous FcγRIIa-R/H131 individuals, respectively (Bredius *et al.,* 1993).

1.2.5.2 Molecular Methods

Polymerase Chain Reaction (PCR) amplification is used to distinguish between the FcγRIIa- H131 and R131 and FcγRIIIb-NA1 and NA2 genotypes (De Haas *et al.*, 1995; Osborne *et al.*, 1994).

PCR is an *in vitro* method for nucleic acid synthesis by which a target DNA is exponentially replicated (Saiki *et al.*, 1985; Mullis *et al.*, 1986). It uses a thermostable DNA polymerase isolated from *Thermus acquaticus, Taq* polymerase and two oligonucleotide primers which flank the target DNA sequence to be amplified. The reaction involves repeated cycles of heat denaturation of DNA, annealing of primers to their complementary sequences at a lower temperature, and extension of the annealed primers with the polymerase. The primers hybridise to the opposite strands of the target DNA and are oriented (3' ends pointing towards each other) so that DNA synthesis by the polymerase proceeds across the region between the primers. The extension products are complementary to and capable of binding primers, therefore successive cycles of amplification result in the doubling of the target DNA synthesized in the previous cycle.

Polymerase Chain Reaction uses equimolar concentrations of the two primers. These primers are usually designed to hybridise to conserved regions of the genome. Primers are also designed such that the annealing temperatures in the PCR reaction are as high as possible to ensure that they are specific during amplification (Thein & Wallace, 1986). Under standard conditions the annealing temperature in a reaction should be 5°C lower than the melting temperature (Tm) of the primers and this is determined using the following formula (Thein & Wallace, 1986);

$$Tm = [4\ (G+C) + 2\ (A+T)]$$

where G, C, A, and T are guanine, cytosine, adenine and thymine, respectively. The standard PCR amplification protocol amplifies most target DNA, however optimal performance is sought by varying most parameters and conditions for each new amplification (Innis & Gelfand, 1990)

The standard reaction mix contains 1X PCR reaction buffer, 200μM each of deoxyribonucleotides (dATP, dCTP, dGTP, dTTP), 2.5 units of *Taq* DNA polymerase, 0.5μM each of the forward and reverse primers, and 1ng-1μg of template DNA. The temperature cycling is performed using a programmable thermal cycler, programmed to carry out the repeated cycling of denaturation at between 94°C and 96°C, primer annealing at a range of 58°C - 60°C and primer extension at 72°C.

The sizes of PCR products are estimated by comparison with the mobility of standards of known molecular weights on agarose gel. In principle, the mobility of a DNA molecule is related to its size (Sambrook *et al*, 1989). The larger the molecule, the lower the rate of migration. The rate of migration of DNA molecules on agarose gels is inversely proportional to the logarithms of the molecular weights as expressed below:

$$D = a - b (\log M)$$

where D is the distance moved by the DNA molecule on the agarose gel, M is the molecular weight of the DNA and a and b are constants.

PCR method for identification of the FcγRIIa allotypes is done by first amplifying the FcγRIIa gene using gene –specific primers (Osborne *et al.,* 1994). The sense

primer used is upstream to the polymorphism encoding amino acid 131 in the second extracellular domain and does not distinguish between the genes for FcγRIIa, b or c. The antisense primer is located downstream of the polymorphism in the transmembrane domain and contains nine bases on the 3' end of the primer which is unique to the FcRIIa gene

FcγRIIa genotyping is accomplished by allele-specific polymerase chain reaction. Two more allele specific PCR reactions are carried out on the PCR product obtained from the first round of PCR described above. Both reactions will utilize a common antisense primer located downstream to the polymorphism, and one of the two allele-specific primers. The two allele-specific primers were designed with the 3' base complementary to the allovariable base:

H131-specific: 5'-GAA AAT CCC AGA AAT TCT CCC A-3'
R131-specific: 5'-GAA AAT CCC AGA AAT TCT CCC G-3'.

FcγRIIIb genotyping can also be done using the polymerase chain reaction with primers designed specifically for the NA1 and NA2 allotypes (De Haas *et al.,* 1995).

Another means by which the FcγRIIa allotypes can be differentiated is by allele specific oligonucleotide (ASO) hybridisation. This method is an established technique used to distinguish single or multiple nucleotide polymorphisms (Saiki *et al.*, 1985).

CHAPTER 2

MATERIALS AND METHODS

2.1. CHEMICALS AND REAGENTS

The reagents for PCR, 10X PCR Buffer, magnesium chloride (50mM MgCl2), *Taq* DNA polymerase, deoxyribonucleoside triphosphates: deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), deoxythymidine triphosphate (dTTP) and molecular weight marker (123 basepair ladder), were purchased from Sigma Chemical Company, St Louis U.S.A, and GIBCO BRL (Life Technologies U.S.A). Saponin, absolute ethanol, sodium chloride, sodium hydroxide, potassium chloride, sodium citrate, sodium phosphate, silver nitrate, potassium phosphate, mineral oil, chelating resin, bromophenol blue, sucrose, agarose (molecular biology grade) and ficoll, were also purchased from Sigma Chemical Company, St Louis, U.S.A. All the oligonucleotide primers, and cell lines, K562 and U937, which were used as controls, were purchased from Alta Bioscience, University of Birmingham, U.K. Tris (hydroxymethyl) aminomethane, ethylene diamine tetra acetate, (EDTA) disodium salt, acetic acid and ethidium bromide were obtained from Fluka AG Granite Chemika, Switzerland. Orange G was obtained from Kanto Chemical Company Ltd., Japan.

2.1.1 Preparation of Buffers and Solutions

The various buffers and solutions used were prepared as described in Appendix I

2.2 BLOOD SAMPLE COLLECTION

2.2.1 The study areas

The first part of the work, which involved collecting blood samples from children who were asymptomatic for malaria to serve as controls, was conducted in Dodowa,

the capital town of the Dangbe West District of Ghana. The Dangbe West District is situated between longitudes 0° 5' E and 0° 20'E and latitudes 5° 40' and 6° 19' N. Dodowa is predominantly a farming community with a population of about 6,500 inhabitants (Appawu & Baffoe-Wilmot, 1997). The vegetation in Dodowa can be described as coastal forest, which lies between the coastal savannah to the south and secondary forest areas to the north. A permanent stream with forest gallery along the banks runs through the town. However as a result of deforestation very little of the original forest is left. Rainfall is seasonal with two peaks occurring in June and September (Appawu & Baffoe-Wilmot, 1997). Dodowa is known to be a hypoendemic area for malaria. Although a few cases of *P. malariae* is seen, most of the cases of malaria are due to *P. falciparum,* which is transmitted mostly by *A. gambiae* s.s. (Appawu & Baffoe-Wilmot, 1997).

The second part of the study, which involved screening for children with clinical malaria, was carried out at the Department of Child Health of the Korle-Bu Teaching Hospital, in Ghana. Although people from all walks of life bring their ailing children to the Department of Child Heath, most of the patients seen daily are children from the immediate environs of the hospital, who are usually children of low- income families.

The suburbs around the hospital are not very well planned as far as social infrastructure is concerned. There are no or very poor drainage systems in the areas, no waste disposal systems in place and poor toilet facilities. During the rainy seasons (May- August), very severe falciparum malaria is one of the major diseases reported at the hospital.

2.2.1.1 Field Sample Collection

An initial cohort of 250 children aged between 1 and 14 years of age were randomly selected at Dodowa. The subjects were asked for their ethnic background and then examined by a medical officer to establish their health status. Venous blood of the individuals were then taken by a qualified personnel onto Whatman No. 5 filter paper and also onto glass slide. The filter papers were air-dried and preserved individually in plastic bags and stored at -20°C until ready to be used whereas the slides were sent to the laboratory for microscopy studies. Seventy-five of the children were selected to serve as controls based on the health status. This study was carried out over a period of 5 months from February to June 2000.

2.2.1.2 Hospital Sample Collection

Patients reporting to the Department of Child Health, Korle Bu Teaching Hospital with malarial–like symptoms, were the subjects used for this study with the approval from the ethics board and informed consent of their parents. A questionnaire was given them to complete (Appendix II).

The patients were first examined by the Medical Officer in charge and thereafter venous blood and urine samples were taken for analysis. The patients presenting malaria-like symptoms were grouped into different clinical categories using the inclusion and exclusion criteria that are stated below in Table 2.1. A medical officer ascertained the clinical status of each patient after the results of the blood and urine analyses had been obtained.

Table 2.1. Inclusion and exclusion criteria used for grouping of patients into different clinical categories.

Clinical category	Inclusion criteria	Exclusion criteria
Cerebral malaria (CM)	Parasitaemia Blantyre coma scale ≤ 3 Duration of coma > 60 mins	Other neurological disease Recent severe head trauma Other cause of coma
Severe malarial anaemia (SA)	Parasitaemia Haemoglobin ≤ 5 g/dl	Blantyre coma scale < 5 at any time Observed convulsions Recent severe bleeding Other cause of anaemia
Uncomplicated malaria (UM)	Parasitaemia Haemoglobin ≥ 8 g/dL Full consciousness	Blantyre < 5 at any time Reported or observed convulsions Respiratory distress "Coco cola" urine Other major organ failure
Respiratory distress (RD)	Parasitaemia At least one of the following Abnormally deep (breathing) Alar flare Use of accessory respiratory muscles e.g supraclavicular, suprastenal Recessions Chest recessions	Exclusion of other causes of respiratory distress (e.g. pneumonia)

2.3 Blood Analysis

2.3.1 Parasitaemia

Thin blood films were stained with 5% Geimsa solution and the films were examined by an experienced malaria microscopist using the standard examination protocol of 200 fields per film.

2.3.2 Haemoglobin level Estimation

Whole blood was collected into a vacutainer containing K_3EDTA and the haemoglobin level determination was performed using the automated Sysmex blood analyser.

2.3.3 Glucose Estimation

An aliquot of 2ml of Glucose oxidase was mixed with 20µl blood plasma and incubated at room temperature for 25 minutes. The colour obtained was read off a Microlab 2000 analyser to estimate the blood glucose level.

2.3.4 Sickling Status

A drop of venous blood was mixed with a drop of 2% sodium metabisulphite ($Na_2S_2O_5$) on a slide and covered with a cover-slip. This was then left in a humid chamber for at least 30 minutes and examined under a microscope for the sickle cells or the holy leaf appearance.

2.4 Urinalysis

The urine from the patients was collected in a transparent tube and visually examined to see if the colour was dark as the coca cola drink or not.

2.5 Extraction of Human DNA from Filter Paper Blood Blot

Human DNA was isolated from the blood blot on filter paper as described by (Wooden *et al.,* 1993). Briefly a piece of the blood spot was cut out of the filter paper with a sterile blade and placed into a 1.5ml eppendorf tube containing 0.5% saponin in 1 X Phosphate Buffered Saline (PBS) solution. This was then incubated overnight at 4°C and thereafter spun at 13 000 rpm using a Kubota1120 microcentrifuge for 1 minute. The supernatant was poured off and the filter paper then washed twice in 1ml of 1 X PBS. The filter paper was transferred into a fresh tube and 150µl sterile double distilled water and 50µl of 20% chelex solution were added and the mixture boiled for 8mins. It was then centrifuged for 5 minutes to pellet the chelex and debris. The supernatant containing the DNA was transferred into a fresh sterile tube and stored at -20°C until ready to use for PCR.

2.6. Determination of FcγRIIa Allotypes using Allele-Specific PCR

This method involved two rounds of PCR. The FcγRIIa gene was amplified as described by Osborne *et al.,* (1994), using gene-specific primers. For the initial PCR, 5 µL of extracted DNA were added to a 20µl reaction mixture containing 2mM $MgCl_2$, 50mM KCl, 20mM Tris-HCl, pH 8.3, 0.1 mg/ml of gelatin, 200 µM of each of the four deoxyribonucleotides, 0.5µM of each oligonucleotide primer, P52 and P63 (Table 4) and 0.5U of *Taq* DNA polymerase.

The PCR cycling conditions were 1 cycle of 95°C for 3 mins; 60°C for 45 s; 72°C for 4 mins, followed by 35 cycles of 95°C for 1 min; 55°C for 45 s; 72°C for 2 min: and ended with a final cycle of 72°C for 10 min.

Two further PCR reactions which are allele-specific were carried out on the PCR product obtained from the first round of PCR described above. Both reactions utilized a common antisense primer, R2A (Table 2.2) and one of the two allele-specific primers, R2R or R2H (Table 2.2). The PCR reactions were in total volumes of 25µl containing 0.5µl of the PCR product, 0.1µM sense and antisense primers, 2.75mM $MgCl_2$, 50mM KCl, 20mM Tris-HCl (pH 8.3), 0.1mg/mL gelatin, 200µM each of deoxyribonucleotides and 0.5U *Taq* DNA polymerase. The cycling program was set to the following parameters: 95°C for 5 min; 35 cycles of 95°C for 15s; 58°C for 30 s; and 72°C for 30 s, and a final extension step of 72°C for 10 min.

Table 2.2 DNA sequences of oligonucleotide primers used in genotyping

Primer	**DNA Sequence (5'-3')**	**Size (bp)**	**Melting Temp (°C)**
P52	CAAGCCTCTGGTCAAGGTC	19	55
P63	GAAGAGCTGCCCATGCTG	18	57
R2A	CTAGCAGCTCACCACTCCTC	20	55
R2H	GAAAATCCCAGAAATTCTCCCA	22	60
R2R	GAAAATCCCAGAAATTCTCCCG	22	62
NA1A	CATGGACTTCTAGCTGCACCG	21	61
NA1S	CAGTGGTTTCACAATGTGAA	20	52
NA2S	CTCAATGGTACAGCGTGCTT	20	56
NA2A	CTGTACTCTCCACTGTCGTT	20	49

2.7 Determination of FcγRIIIb Genotypes by PCR

FcγRIIIb genotyping was performed using the PCR amplification of the genomic DNA. The reaction to determine the NA1 gene was done in a 25μL which contained 5μL of extracted DNA, 0.8μM each of the sense and antisense primers, NA1S and NA1A (Table 2.2), 2mM $MgCl_2$, 20mM Tris-HCl (pH 8.3), 50mM KCl, 0.1mg/ml gelatin 200μM each of the four deoxyribonucleotides and 0.5U *Taq* DNA polymerase. The mixture was thoroughly mixed and centrifuged briefly at 10 000 rpm in a Kubota 1120 centrifuge.

The temperature profile for this reaction was 95°C for 5 min, followed by 10 cycles of 95°C for 1 min; 60°C for 1.5 min; 72°C for 2.5 min; and then 25 cycles of 95°C for 1 min; 57°C for 1 min; 72°C for 1 min. A 10 min extension at 72°C concluded the reaction.

For the amplification of the FcγRIIIb-NA2 DNA, a reaction mixture containing the following was prepared. 20mM Tris-HCl, 50mM KCl, 0.1mg/ml gelatin, 5μl of extracted DNA, 0.1μM each of the sense and antisense primers, NA2S and NA2A (Table 2.2), 200μM each of the four deoxyribonucleotides and 0.1 U of *Taq* DNA polymerase. The cycling parameters used were 95°C for 5 min, followed by 35 cycles of 95°C for 1 min; 46°C for 1.5 min; 72°C for 2.5 min, and thereafter 72°C for 10 min.

2.8 Analysis of PCR Products for Identification and Phenotyping

Following the PCR, the products were electrophoresed on a 1.5% agarose gel and stained with 0.5μg/ml ethidium bromide. One micro litre of bromophenol blue (5X)

or Orange G (5X) gel loading dyes was added to five micro litres of each sample. The gel was prepared and run with 1X TAE buffer using either a midi or maxigel system (BIORAD). The gels were run at 80V for one hour, visualised and photographed over a UV transilluminator (UPC, USA), at short wavelength using a Polaroid camera (IBI 46 400) fitted with an orange filter and a Polaroid type 667 film. The sizes of the PCR products were estimated by comparison with the mobility of standards of known DNA molecular sizes.

2.9 Statistical Analysis

The statistical softwares used for the analysis were SPSS 10.00 for Windows and Epi Info 6 for Dos. Fcγ RIIa allotypes distribution (H/H131, H/R131, R/R131) and allele frequencies (H, R) were analysed by applying the χ^2 test. To reject the null hypothesis, a probability of 0.05 was used. The FcγRIIa polymorphism was then analysed as a dichotomous variable comparing the homozygous FcγRIIa-H/H131 with the homozygous FcγRIIa-R/R131 and heterozygous FcγRIIa-H/R131 allotypes using the χ^2 test in controls (non malarious patients) and malaria subjects presenting with different phases of the disease.

The same analysis was done for the FcγRIIIb genotype distribution (NA1/NA1, NA2/NA2 ,NA1/NA2 and the null genotype) and allele frequencies (NA1, NA2).

CHAPTER 3

RESULTS

3.1 STUDY POPULATION

The details of the demographics and PCR data are given in Appendix III

3.1.1 Control Group

Seventy-five children out of the 250 randomly selected individuals from Dodowa who were without malaria parasites formed the healthy controls. Out of these 38, (50.7%) were males and 37 (49.3%) were females. The age distribution of the control group is shown in Figure 3.1. Their ages ranged from 1-13 years with a mean of 7.4 (standard deviation = ± 3.07). The ethnic distributions of these children were as follows: 13 (17.3%) were Northerners, 21 (28%) were Ewes, 30 (40%) were Gas and 11 (14.7%) were Akans

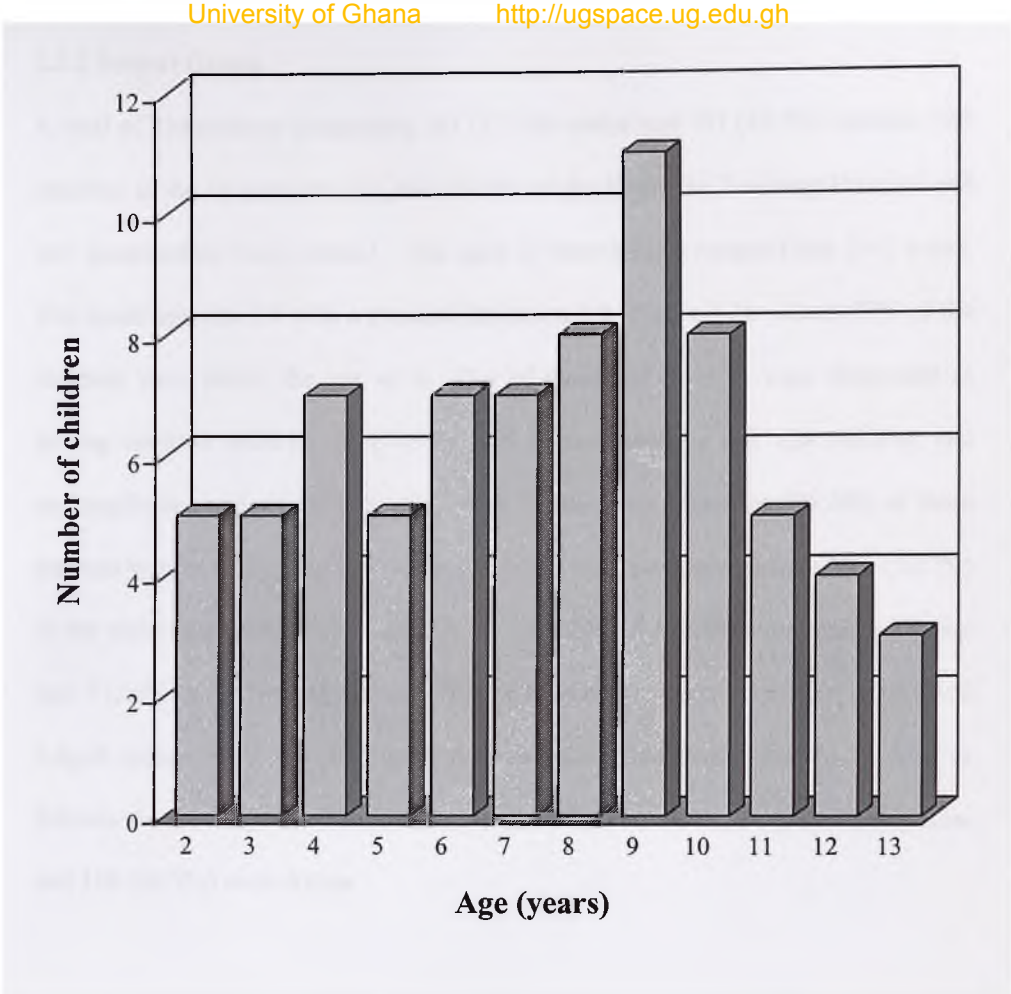

Figure 3.1. Age distribution of the control group

3.1.2 Patient Group

A total of 254 patients comprising 147 (57.9%) males and 107 (49.3%) females who reported at the Department of Child Health of the Korle Bu Teaching Hospital and had parasitaemia were studied. The ages of the children ranged from 1 -13 years. The mean age was 3.8 with a standard deviation 2.9 (Figure 3.2). About 77% of the children were below the age of 6. Out of these, 54 (21.3%) were diagnosed as having cerebral malaria, 76 (29.9%) had severe anaemia and 124 (48.8%) had uncomplicated malaria (Figure 3.3). Two hundred and forty-two (95.3%) of these patients were sickling negative, while 12 (4.7%) were positive. Eighty-three (32.7%) of the patients presented with 'coca cola' urine, 39 (15.4%) with respiratory distress and 7 (2.8%) were hypoglycaemic. The mean haemoglobin level of the patients was 6.6g/dl (range: 0.4g/dl 14.0 g/dl) (Figure 3.4). The ethnic distribution was as follows: 64 (25.2%) were Northerners, 40 (15.7%) were Ewes, 41 (16.1%) were Gas and 109 (42.9%) were Akans.

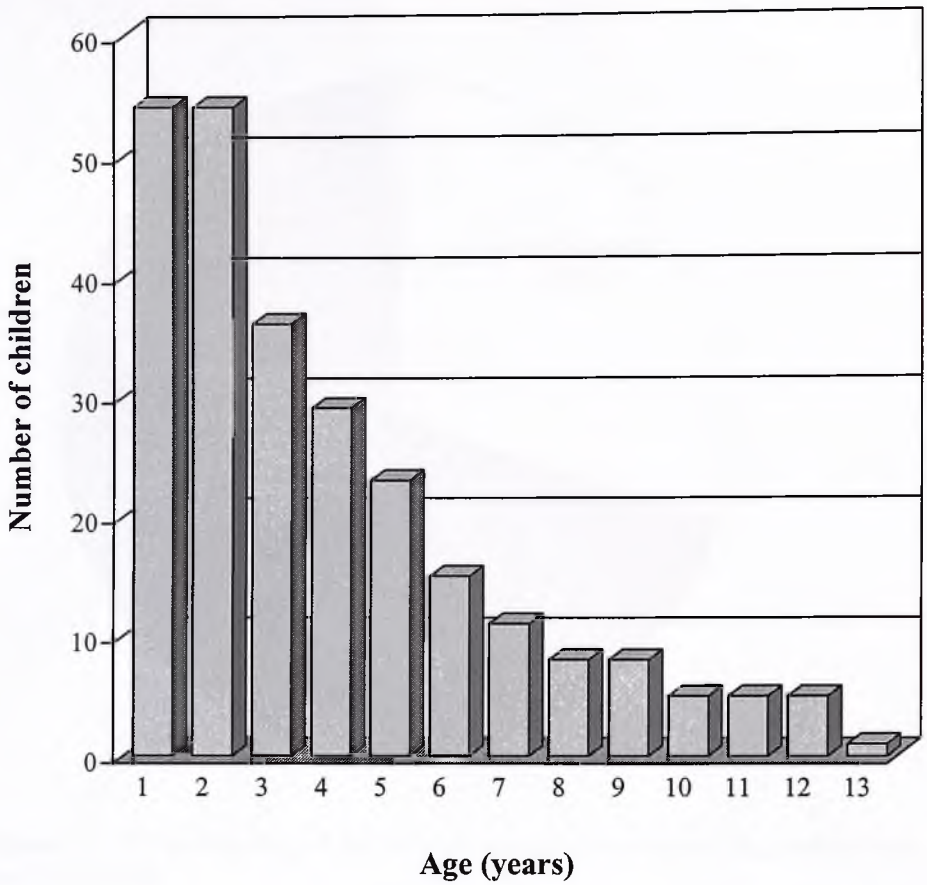

Figure 3.2. Age distribution of malaria patients recruited at the Korle Bu Teaching Hospital.

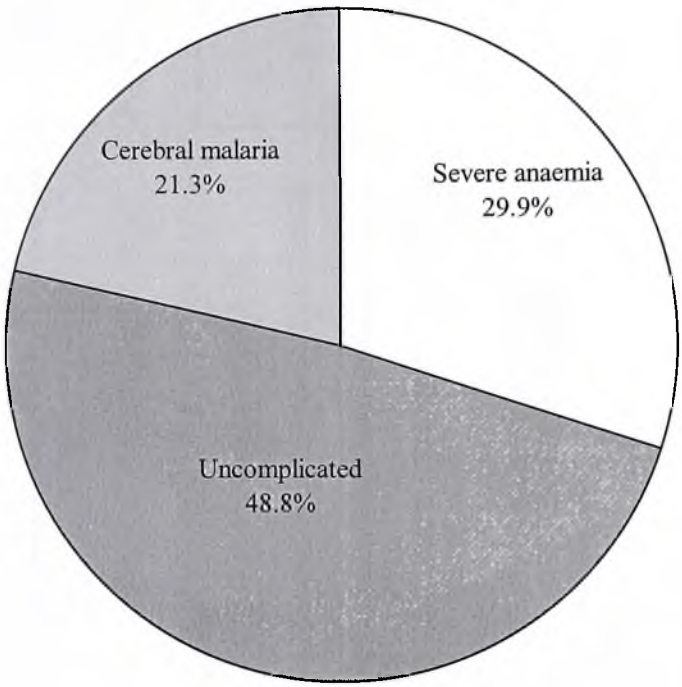

Figure 3.3. The distribution of the different malaria presentations diagnosed among the 254 patients.

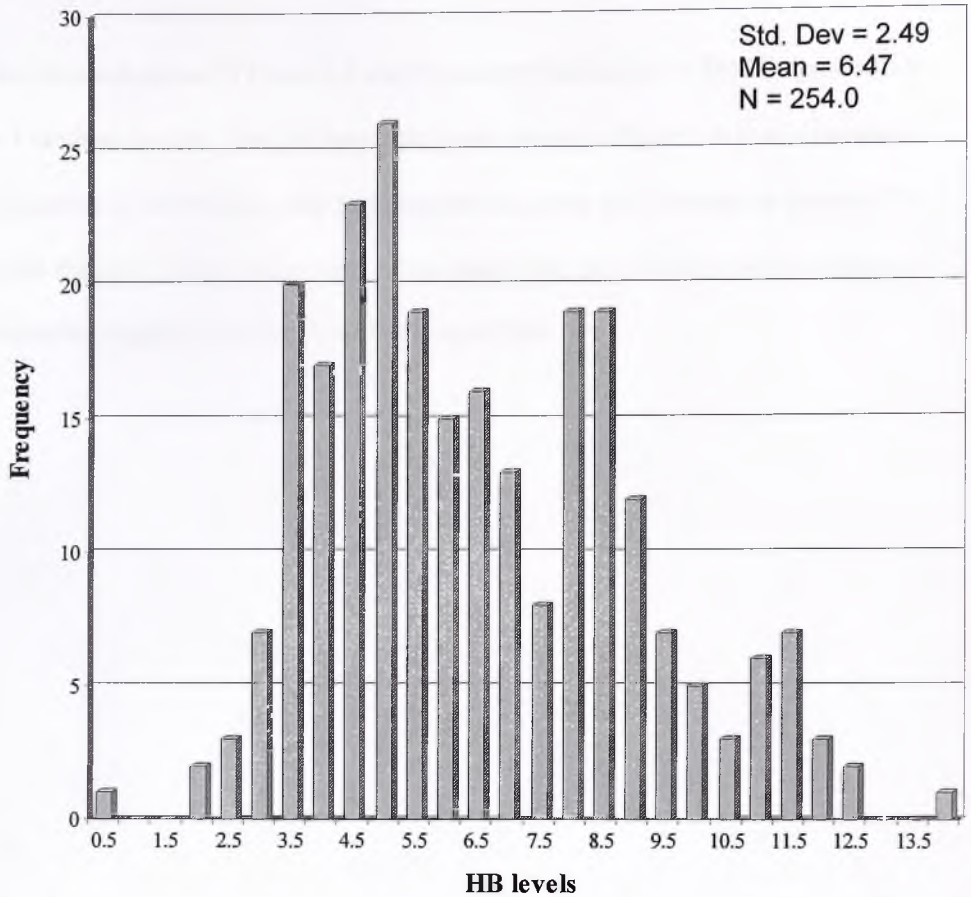

Figure 3.4. The haemoglobin profile of the patient group.

The mean haemoglobin value seen in the patients was 6.5 g/dl. 38% of them recorded Hb levels below 5 g/dl which is the cut off point for severe malaria anaemia.

3.2 FcγRIIa Genotype Distribution

The electrophregram in Figure 3.5 shows the amplified region of FcγRIIa gene which is 1 kilobase in size. The 290 base pair bands shown in Figure 3.6 is an example of the portion of the FcγRIIa gene that contains the amino acid histidine at position 131 while Figure3.7 shows an example of the bands obtained when the polymorphic site containing arginine instead of histidine is amplified.

Figure 3.5 An example of electrophoregram of PCR amplification of the human FcγRIIa gene using P52 and P63.

Lane M contains the 123 molecular weight marker,

Lane 6 has the negative control

Lanes 1-5 and 7-10 are PCR products of the FcγRIIa gene.

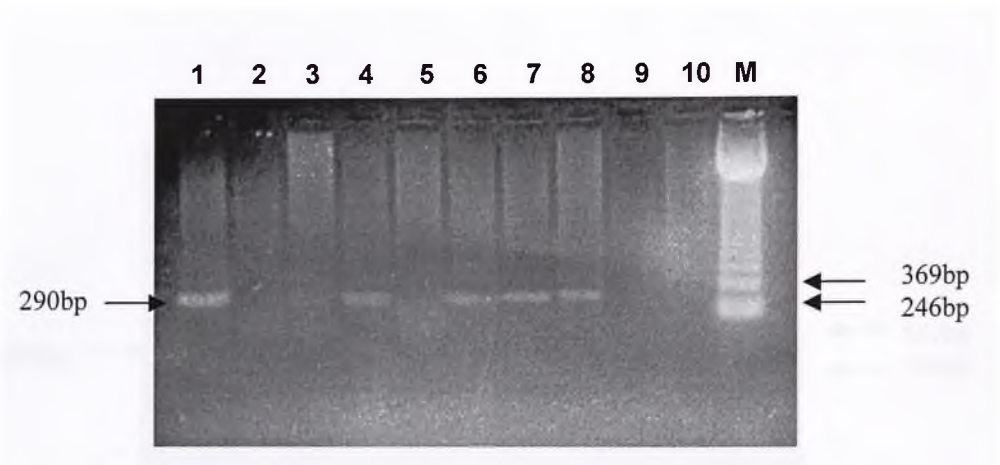

Figure 3.6. An example of electrophoregram of PCR products using primers R2A and R2H that amplifies the region with FcγRIIa polymorphism.

Lane M is the 123 molecular weight marker,

Lanes 2, 9 and 10 indicating the absence of the H allele, can only be typed as R/R131.

Lanes 1,3,4, 6, 7 and 8 have the diagnostic size of 290bp indicating the presence of the H allele and therefore can either be the H/H131 or H/R131 genotype.

Figures 3.7 An example of electrophoregram of PCR amplification using primers R2A and R2R that amplify the polymorphic region of the FcγRIIa gene.

Lane M is the 123 molecular weight marker.

Lanes 1,2, 4, 6,7 and 9 have the expected band size of 290bp, indicating the presence of the R allele, and can therefore be either the R/R131 or H/R131 genotype.

Lanes 3 and 10, indicating the absence of the R allele, can only be H/H131.

3.2.1 FcγRIIa Genotype Distribution in the Control Group

Fifteen (20%) out of the total 75 healthy children were H/H131 genotype, 44 (58.7%) were H/R131 and 16 (21.3%) were R/R131. Among the 13 Northerners in this group, 2 (15.4%) were H/H131, 3 (23.1%) were R/R131 and 8(61.5%) were H/R131 genotype. Five (23.8%) out of 21 Ewes were H/H131, 6 (28.6%) and 10 (47.6%) were R/R131 and H/R131, respectively. Within the Ga population, 6(20%) were H/H131, 5 (16.7%) were R/R131 and 19 (63.3%) were H/R131. Among the Akans, 2 (18.2%) were H/H131, another 2 were R/R131 and 7 (63.6) were H/R131.

3.2.2. FcγRIIa Genotype Distribution in the Patient Group

Out of a total of 254 patients studied, 28 (11%) were of the H/H131 genotype, 147 (57.9%) were H/R131 while 79 (31.1%) were R/R131. Among the 64 Northerners, 5 (7.8%) were H/H131, 40 (62.5%) and 19 (29.7%) were H/R131 and R/R131 respectively. Among the Ewe patients 6 (15%) were H/H131, 25 (62.5%) and 9 (22.5%) were H/R131 and R/R131 respectively. Of the 41 Ga patients, 4 (9.8%) were H/H131, 22 (53.7%) were H/R131 and 15 (36.6%) were R/R131. Of the 109 Akan patients 13 (12%) were H/H131, 60 (55%) were H/R131 and 36 (33%) were R/R131.

3.2.3 Overall Relationship between FcγRIIa Genotype and Ethnicity

Out of a total of 329 children recruited for this study, 77 (23.4%) were Northerners, 61 (18.5%) were Ewes, 71 (21.6%) were Gas and 120 (36.5%) were Akans. The genotype distribution found within the different tribes is shown in Table 3.1. On the whole, there was no significant association observed between this genotype and ethnicity (χ^2=3.25, P=0.77). Within the control group, statistical analysis revealed no significant association between the different genotypes and ethnic origin

(H/H131, (χ^2= 3.751, P=0.29; H/R131, (χ^2 =2.919, P =0.40; R/R131, (χ^2=5.398, P =0.15). Similarly no significant association was found between FcγRIIa genotype and ethnic origin of patients (H/H131, (χ^2=1.475, P=0.68; H/R131, (χ^2=1.362, P=0.71; R/R131, (χ^2=2.205, P=0.53).

Table 3.1 Overall percentage distribution of the FcγRIIa genotypes within the different ethnic groups

	FcγRIIa genotypes (%)		
Ethnic Group	H/H131	H/R131	R/R131
Akan	12.5	55.8	31.7
Ga	14.1	57.7	28.2
Northerner	9.1	62.3	28.6
Ewe	18.0	57.4	24.6

3.2.4 Distribution of FcγRIIa Genotype of the Control and Patient Groups

The Fcγ RIIa genotypes for the patient and control groups were distributed as shown in Figure 3.8. No significant difference was observed in the overall genotype distribution between the patients and controls (χ^2 =5.49 P=0.06). However the differences between the FcγRIIa-R/R131 genotype distribution in the patients and controls was significant (χ^2 =11.686, P=0.003). No significant differences were observed between the number of children possessing the H/R131 (χ^2 =12.157, P=0.07) and H/H131 (χ^2 =0.269, P=0.87) in both the patient and control groups. The genotype distribution within the various ethnic groupings in both the control and patient groups is shown in Table 3.2

Table 3.2 FcγRIIa genotype percentage distribution among the different ethnic groups studied within the control and patient groups

		Fc□IIa genotype (%)		
Ethnic group		H/H131	H/R131	R/R131
Akan	Controls	18.2	63.6	18.2
	Patient	12.0	55.0	33.0
Ga	Controls	20.0	63.3	16.7
	Patient	9.8	53.7	36.6
Northener	Controls	15.4	61.5	23.1
	Patient	7.8	62.5	29.7
Ewe	Controls	23.8	47.6	28.6
	Patient	15.0	62.5	22.5

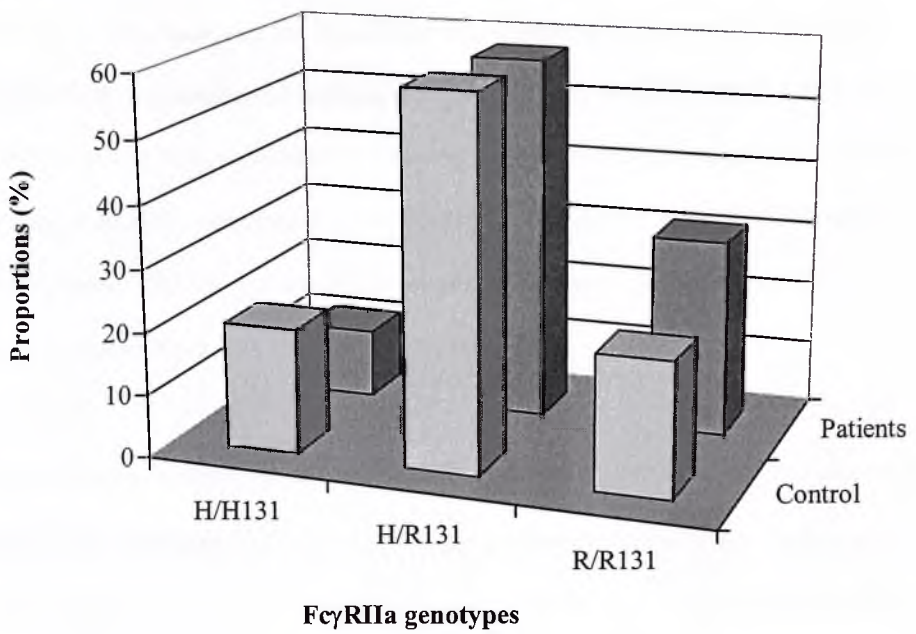

Figure 3.8. Frequency distribution of the FcγRIIa genotypes within the control and patient groups. In both groups the H/R131 genotype was very dominant forming more than 50% for each group. The percentage of patient group found to be homozygous H/H131 were considerably lower than those in the control group. Conversely there were more homozygous R/R131 in the patient group than in the control group.

3.3 Relationship between FcγRIIa Genotype and Disease Status

The results obtained when the different malaria cases and their FcγRIIa genotype frequencies were analysed for association are illustrated in Figure 3.9. Of the 124 patients with uncomplicated malaria, 18 (14.5%) were H/H131, 80 (64.5%) were H/R131 and 26 (21%) were R/R131. Among the 54 patients diagnosed with cerebral malaria, 3 (5.6%) were found to be H/H131, 35 (64.8%) were H/R131 and 16 (29.6%) were R/R131. For the 76 severe anaemia cases, 7 (9.2%) were H/H131, 32 (42.1%) were H/R131 and 37 (48.7%) were R/R131.

The association between the overall FcγRIIa genotype distribution and malaria was found to be significant (χ^2 =25.31, P=0.000) according to this study. While there was no significant association found between uncomplicated malaria and any of the FcγRIIa genotypes, the association between severe anaemia and FcγRIIa-R/R131 and FcγRIIa-H/R131 genotypes was significant; but no association was found between severe anaemia and FcγRIIa-H/H131. Furthermore, there was significant association found between cerebral malaria and FcγRIIa-R/R131, but there was no association found with FcγRIIa-H/R131 and FcγRIIa-H/H131 genotypes.

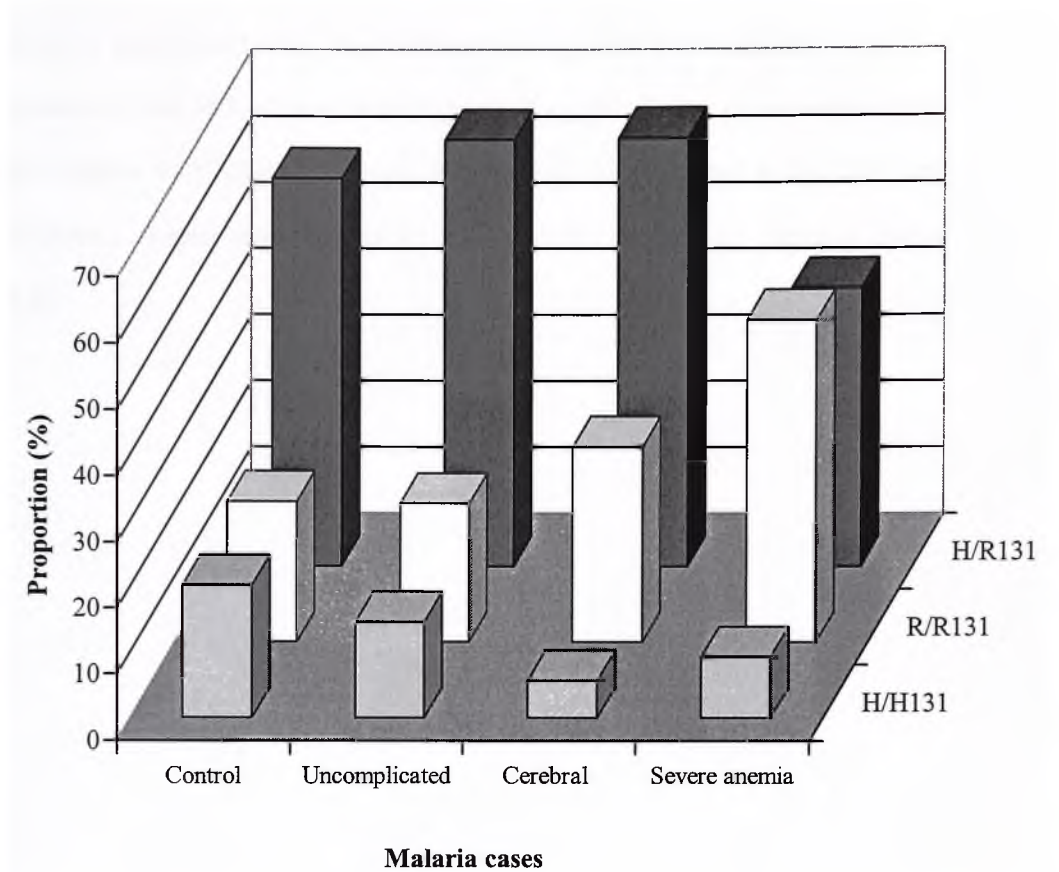

Figure 3.9. **Relationship between FcγRIIa and disease status**

The differences in the genotype distribution between the controls and patient groups gives an indication that the FcγRIIa-R/R131 puts an individual at risk while the FcγRIIa-H/H131 confers protection against malaria. The H allele in the heterozygote state is seen to offer protection against severe malaria anaemia. This is however not the case in cerebral malaria or uncomplicated malaria.

3.4 FcγRIIIb Genotype Distribution

The NA2 primer set amplified a 169bp DNA fragment while the expected size for the NA1 primers is 141bp. An individual testing positive for both NA1 and NA2 primers is typed as NA1/NA2 heterozygous. If an individual tests positive for NA1 but negative for NA2 and vice versa, then that individual is typed as NA1/NA1 and NA2/NA2, respectively. Results for the FcγRIIIb Genotype are shown in Figure 3.10.

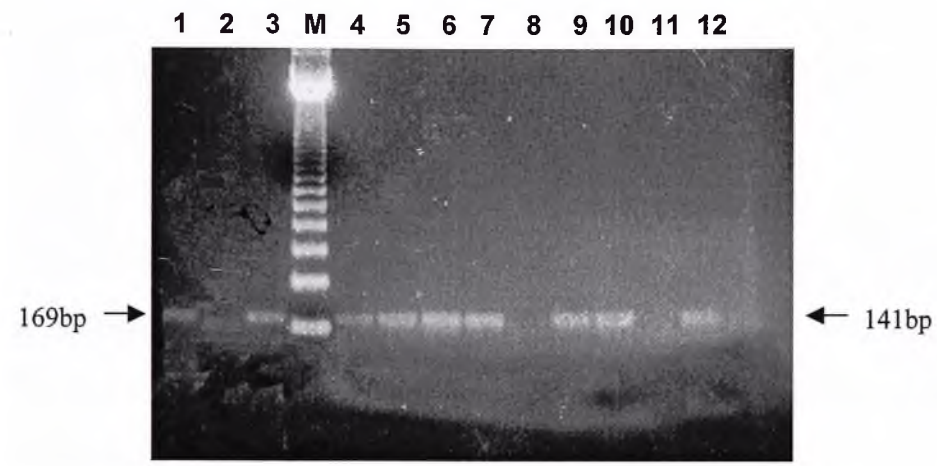

Figure 3.10 An example of electrophoregram of PCR products using primer sets for NA1 and NA2 which amplify the region of the FcγRIIIb polymorphism.

Lane M is 123 molecular weight marker.

Lanes 1 and 3 have the diagnostic size of 169bp indicating the presence of the NA2 allele and can therefore be either NA2/NA2 or NA1/NA2.

Lane 2 indicating the absence of the NA2 allele can only be NA1/NA1 or Null.

Lanes 4,5,6,7,9,10 and 12 having the diagnostic size of 141bp indicate the presence of the NA1 allele and can therefore be NA1/NA1 or NA1/NA2.

Lane 11 indicating the absence of the NA1 allele can either be NA2/NA2 or Null.

Lane 8 is the negative control.

3 4.1 FcγRIIIb Genotype Distribution in the Control Group

The FcγRIIIb genotype frequency distribution within the control group is shown in Figure 3.11. Of the 75 children screened, 12 (16%) were NA1/NA1, 24 (32%) were NA2/NA2, 34 (45.3%) were NA1/NA2 and 5 (6.7%) possessed neither of these genes and were categorized as 'Null'.

Of the 13 Northerners in this group one (7.7%) was found to be NA1/NA1, 6 (46.2%) were NA2/NA2, 5 (38.5%) were NA1/NA2 and one was Null. Among the 21 Ewes in the group one (4.8%) possessed the NA1/NA1 genotype, 7 (33.3%) were NA2/NA2, 10 (47.6%) were NA1/NA2 and 3 (14.3%) were null. Five (16.7%) of the 30 Gas were NA1/NA1, 9 (30%) were NA2/NA2 and 16 (53.3%) were NA1/NA2. Out of the 11 Akans in this group, 5 (45.5%) were NA1/NA1, 2 (18.2%) were NA2/NA2 and 3 (27.3%) were NA1/NA2 and 1 (9%) was null.

3.4.2 FcγRIIIb Genotype Distribution in the Patient Group

The results of the study showed that 104 (40.9%) out of the 254 patients were NA1/NA1, 22 (8.7%) were NA2/NA2, 61 (24%) were NA1/NA2, and 67 (26.4%) were Null genotype (Figure 3.11). Among the 64 Northerners, 21 (32.8%) were Null, 25 (39.1%) were NA1/NA1, 3 (4.7%) were NA2/NA2 and 15 (23.4%) were NA1/NA2. Of the 40 Ewes, 9 (22.5%) were Null, 18 (45%) were NA1/NA1, 6 (15%) were NA2/NA2 and 7 (17.5%) were NA1/NA2. Of the 41 Gas, 13 (31.7%) were Null, 15 (36.6%) were NA1/NA1, 2 (4.9%) were NA2/NA2 and 11 (26.8%) were NA1/NA2. Among the 109 Akans, 24 (22%) were Null genotype, 46 (42.2%) as NA1/NA1, 11 (10.1%) as NA2/NA2 and 28 (25.7%) were NA1/NA2.

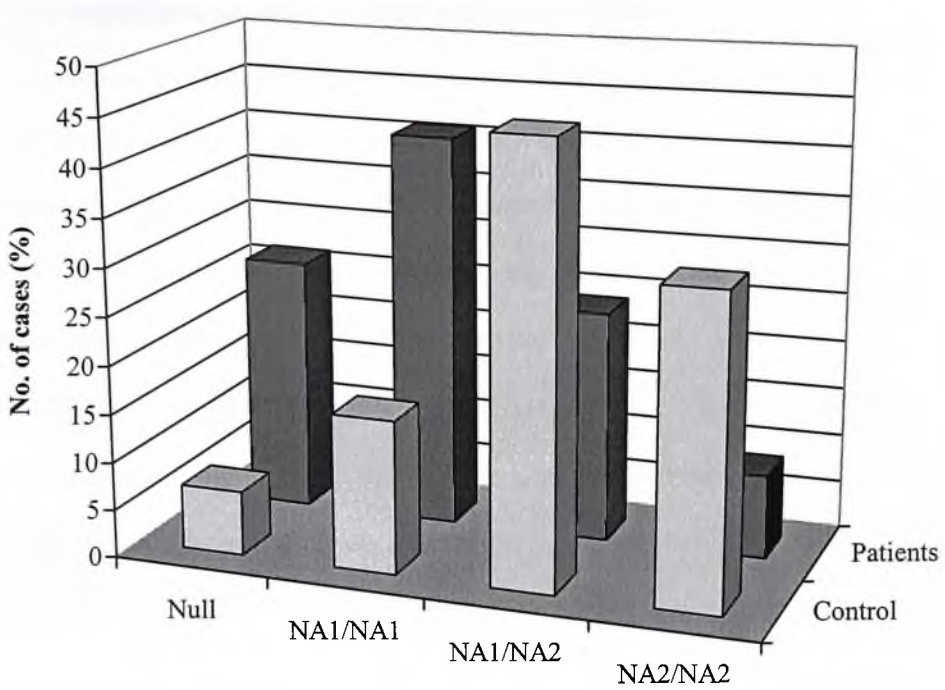

Figure 3.11. Frequency distribution of the FcγRIIIb genotype of the malaria patients and the controls. The trend in the distribution of the various genotypes seems very different between the control and patient groups. While the NA1/NA2 and NA2/NA2 were the predominant genotypes among the control group, it was the NA1/NA1 and Null genotypes that dominated the patient group.

3.4 3. Overall Relationship between FcγRIIIb Genotype and Ethnicity

The genotype distribution found within the different ethnic groups is shown in Table 3.3. On the whole no significant association was found between the patients and controls (χ^2= 11.61, P=0.24). Among the controls, statistical analysis showed that there was no significant association between any of the FcγRIIIb genotype and the ethnic groups (NA1/NA1, χ^2=0.759, P=0.86; NA2/NA2, χ^2=4.333, P=0.23; NA1/NA2, χ^2 =1.146, P=0.76; Null, χ^2 =3.341, P=0.34). Again no significant association was found between this FcγRIIIb genotype distribution and the ethnic groups within the patient group (NA1/NA1, χ^2=4.298, P=0.12; NA2/NA2, χ^2 =0.747, P=0.69, NA1/NA2, χ^2 =4.117, P=0.13; Null, χ^2 =2.194, P=0.33).

3.4.4 Comparison of FcγRIIIb Distribution of the Control and Patient Groups

The FcγRIIIb genotypes for the patient and control groups were distributed as illustrated in Figure 3.11. There was a significant difference observed in the overall genotype distribution between the patients and controls (χ^2 =52.17, P=0.000). Furthermore, there were significant differences in the NA1/NA1 (χ^2 =6.548, P=0.04), NA2/NA2 (χ^2 =21.160, P=0.00) and the Null genotype (χ^2 =7.876, P=0.02) between the patients and controls. No significant differences were found between the patients and controls with the heterozygote NA1/NA2 genotype (χ^2 =0.604, P=0.74).

Table 3.3. FcγRIIIb genotype percentage distribution among the different ethnic groups within the control and patient groups

		Fcg RIIIb genotype (%)			
Ethnic group		NA1/NA1	NA1/NA2	NA2/NA2	Null
Akan	Control	45.5	27.3	18.2	9.0
	Patient	42.2	25.7	10.1	22.0
Ga	Control	16.7	53.3	30.0	0.0
	Patient	36.6	26.8	4.9	31.7
Northener	Control	7.7	38.5	46.2	7.6
	Patient	39.1	23.4	4.7	32.8
Ewe	Control	4.8	47.6	33.3	14.3
	Patient	45.0	17.5	15.0	22.5

3.5. Relationship between FcγRIIIb Genotype and Disease Status

The distribution of the FcγRIIIb genotype frequencies within the different presentations of malaria is shown in Figure 3.12. Out of 124 patients categorized as having uncomplicated malaria 35 (28.2%) were Null, 43 (34.7%) were NA1/NA1, 9 (7.3%) were NA2/NA2 and 37 (29.8%) were of NA1/NA2. Those presenting with cerebral malaria were 54 and out of these 10 (18.5%) were the Null genotype, 27 (50%) were NA1/NA1, 6 (11.1%) were NA2/NA2 and 11 (20.4%) were NA1/NA2. Among the 76 patients who were classified as having severe anaemia, 22 (28.9%) were Null, 34 (44.7%) were NA1/NA1, 7 (9.2%) were NA2/NA2 while 13 (17.1%) were NA1/NA2.

Statistical analysis revealed that the overall FcγRIIIb distribution was significantly associated with the disease malaria (χ^2=60.45, P=0.000). The individual FcγRIIIb genotypes were all associated with malaria as a disease but not with the specific categories. The association between FcγRIIIb-NA1/NA1 genotype and malaria was quite significant (χ^2=7.147, P=0.003) and likewise the FcγRIIIb-NA1/NA2 genotype (χ^2=11.160, P=0.001) compared to the control. FcγRIIIb-NA2/NA2 genotype also showed a significant relationship with malaria (χ^2=9.239, P=0.04). The association between the null genotype and malaria (Figure 3.13) was also found to be significant (χ^2=7.876, P=0.02). There was however, no significant association found between the FcγRIIIb genotype frequency and the presentation of 'coca cola' urine (χ^2=2.692, P = 0.44) and also the presentation of respiratory distress (χ^2 = 3.555, P = 0.737).

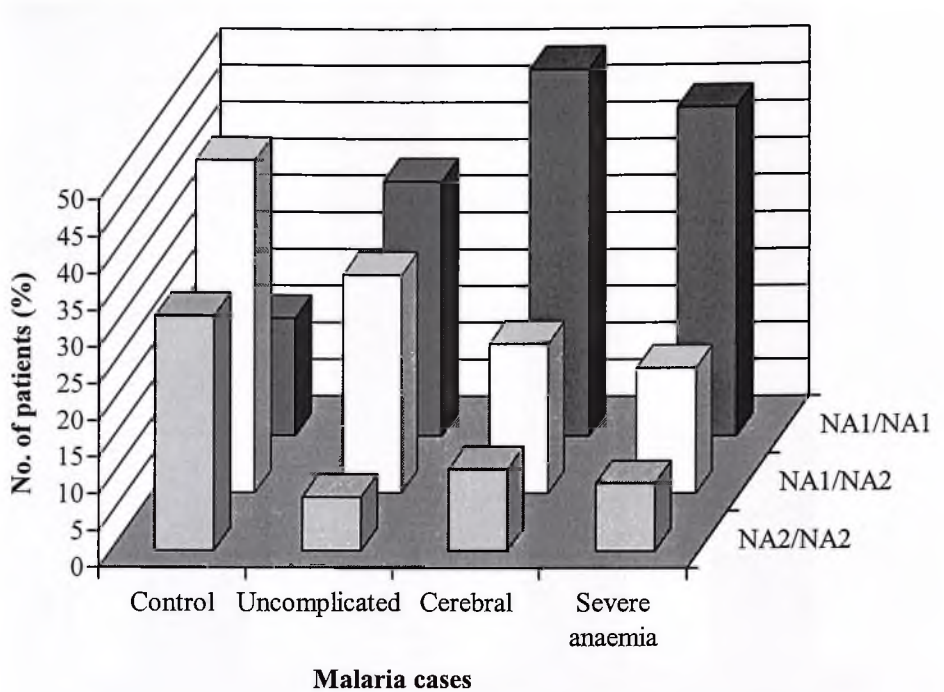

Figure 3.12. Frequency distribution of the FcγRIIIb genotypes within the different disease categories.

The relationship between FcγRIIIb and malaria shows that those with the homozygous NA1/NA1 genotype are more likely to be at risk while NA2/NA2 seems to lend some protection against malaria.

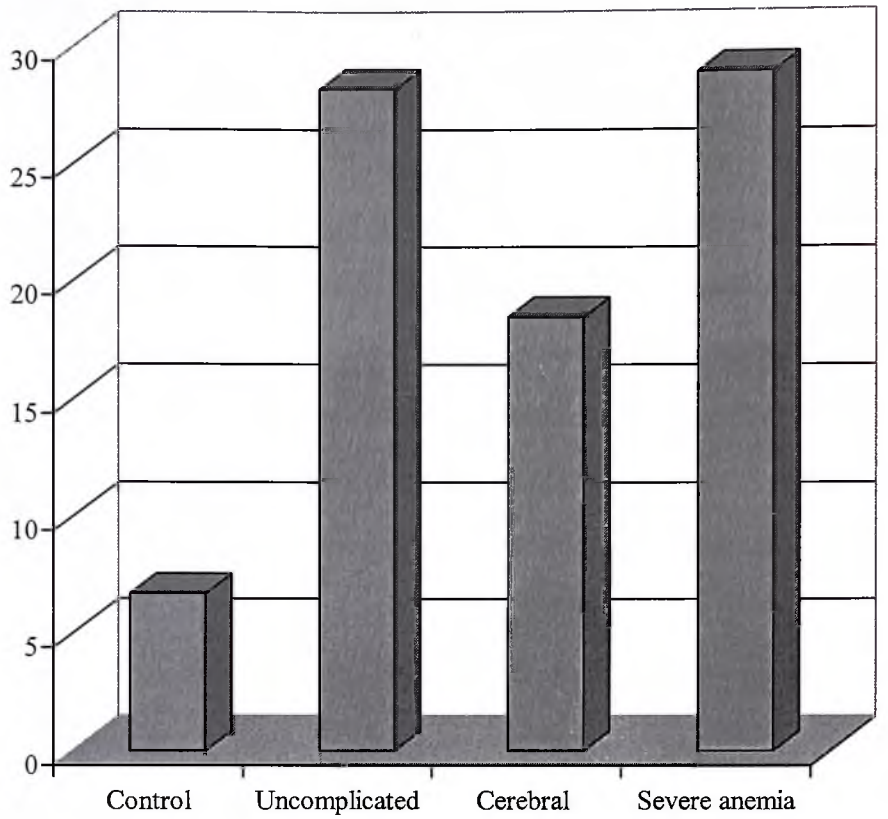

Figure 3.13 Prevalence of the null genotype within the different malaria patient groups

Compared to the controls the null genotype seem to be enhanced within the patient population, especially in the case of uncomplicated malaria and severe anaemia

CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

This present study was set out to find out firstly, whether the distribution of the Fcγ receptors IIa and IIIb was ethnically biased, and secondly to determine if there was any association between the genetic polymorphism of these receptors and the incidence of severe malaria in Ghanaian children. The study also tested the probability of the FcγRIIa, and FcRIIIb polymorphisms, that is, the H-R and NA genotypes being associated with a person's susceptibility to severe malaria.

The homozygous H/H131 for the FcγRIIa receptor was found to be generally under represented among all the tribes, but the distribution was not significantly different when the control population was compared to the patient group (P=0.29). There was however a high representation of the heterozygous H/R131 in all the different ethnic groups, ranging from 47.6% among the Ewes to 63.6% among the Akans in the control group and 53.7% in the Gas to 62.5% in the Ewes within the patient group. Here again, the difference between the two groups was not significant (P=0.68). With regards to the homozygous FcγRIIa-R/R131, there were also no significant differences between the two groups (P=0.63). Even though the distribution of the ethnic groups was not similar between the two study groups, the insignificance of the differences between the overall genotype distribution in the controls and patients validates the use of the subjects from Dodowa as controls for the study.

The heterozygous H/R131 genotype was found to be the dominant genotype being 58.7% and 57.9% among the two study groups. That H/R131 was dominant is not

surprising because this finding compares with the results of a study by Salmon *et al.* (1996) among black African Americans in which 50% of them possessed this same genotype and Shi *et al.* (2001) found 61% of Kenyan children possessing it.

With regards to the disease category and the genotype distribution, three significant associations were found; that the number of patients with R/R131 was significantly higher in the cerebral malaria and severe anaemia groups and H/H131 was significantly lower in the severe anaemia group ($P \leq 0.002$ in all cases). These results suggest that possessing the homozygous FcγRIIa-R/R131 genotype may predispose or increase the susceptibility of an individual to severe anaemia and cerebral malaria.

Although a great deal of work has been done, the pathogenesis of the anaemia associated with *falciparum* malaria remains uncertain. It may well be that the inability of the FcγR/R131 to bind IgG_2 coated parasitized erythrocytes efficiently, delays their immune clearance, thus leading to more parasitized erythrocytes in the human host, thereby resulting in anaemia. There is also the evidence that in severe anaemia, there is reduced monocyte function as a form of immune suppression, and this subsequently leads to reduced Fc-mediated phagocytosis, especially in the case of the less efficient R/R genotype (Ward *et al.,* 1984). This could therefore explain why a large proportion of the patients who were diagnosed as having severe anaemia possessed this genotype. Abdalla and Weatherall (1982) have shown that in severe malarial anaemia, the monocyte erythrophagocytosis of non-parasitized erythrocytes is elevated, and this means that anaemia in acute malaria could be due to opsonization and phagocytosis of both parasitized and non-parasitized red cells. This reasoning, however, does not fully explain the reason for the over representation of

the R/R131 genotype in severe anaemia cases, since the binding capability of the R/R131 genotype of the Fcγ receptor is not as efficient as the H/H131.

In a recent publication, Shi *et al.* (2001), suggested that regardless of its association with increased susceptibility to encapsulated bacterial infections, the frequency of FcγRIIa-R/R131 remains relatively stable in most human populations; implying that it is being selected for. The authors explain that this could be an indication of infections that depend on IgG_3 and IgG_1 but not on IgG_2 to mediate protective immunity, thus providing selective advantage for the poorly IgG_2-binding FcγRIIa-R/R131 genotype. Furthermore, Shi *et al.* (2001) reported that Kenyan children with FcγRIIa-R/R131 were less likely to have repeated high-density *P. falciparum* infection, which suggests that this genotype may rather have a protective effect and that this protective effect may be dependent on IgG1 and/or IgG3 but not IgG_2. However, it must be noted that severe malaria is not solely dependent on parasite load but rather on an inter combination of other factors such as accelerated destruction of non-parasitized erythrocytes and bone marrow dysfunction.

The finding that high proportion of the patients possessing the R/R131 genotype among cerebral malaria cases may be attributed to the inability of this receptor genotype to effectively bind and thus clear the parasites quickly enough. This may therefore lead to more parasitized red blood cells finding their way into the brain tissues where they are sequestered into the microvascular endothelium of the brain cells. The consequences of this sequestration are reduced oxygen and substrate supply, leading to anaerobic glycolysis and lactic acidosis which gives rise to the coma.

There was however no association found between this genotype and uncomplicated malaria and this may be due to the low parasitaemia and therefore the relative inability of the FcγRIIa-R/R131 to bind may not be relevant in the immune clearance of these parasites.

The dominant effect of the R allele in severe anaemia cases is reduced as seen in the number of the heterozygote H/R131. This is not surprising since this genotype is expected to be intermediate in the binding of IgG2 compared to H/H131 homozygous (van de Winkel and Capel, 1993). This raises the possibility that the apparent association of this genotype with malaria may be due to events that occur after antibody binding to monocytes, such as monocyte activation and release of soluble factors. However it remains to be determined whether there is a potential interference between the two genotypes, expressed on the same cell, and / or whether the interference consequently causes a defect of activation and cytokine production of monocytes in the FcγRIIa-H/R131 heterozygotes.

There was significant difference in the number of patients presenting with all disease forms with the homozygous H/H131 genotype ($P \leq 0.01$ in all cases). It indicates that children with the H/H131 genotype are less likely to be at risk from severe malaria than those with R/R131. This would mean that the allotype, H, confers some protection against all forms of the disease. The role of FcγRIIa-H/H131 genotype in malaria infection might be more complex, since this allotype can efficiently bind IgG_2, as well as IgG_1 and IgG_3. Previous *in vitro* studies have shown that ADCI function mediated by IgG_1 and IgG_3 is inhibited by IgG preparation with high IgG_2 concentration (Bouharoun-Tayoun *et al.*, 1995). Indeed, Shi *et al.* (1999) observed that the balance between IgG_1, IgG_2 and IgG_3 is a biologically important factor in the

role of FcγRIIa-H/H131 genotype in malaria infections. Although the H allele confers protection, possessing it in the heterozygous state, does not influence the development of cerebral malaria. It is therefore not dominant in its effect unlike FcγRIIa-R/R131 in severe anaemia.

In a similar study to find the relationship between the FcγRIIa polymorphism and infection in children, H/H131 was found to be over represented, (H/H131 – 64%, H/R131 – 27% and R/R131 – 9%) in black children with sickle cell disease (Norris *et al.*, 1996). Considering the fact that the sickle cell trait is a genetic factor which tends to protect individuals from malaria, it is therefore not surprising that those presenting with severe malaria showed an under representation of the H/H131 genotype. This is also supported by a study by Salmon *et al.*, 1996 among certain Africa Americans with systemic lupus erythematosus (SLE), which has similar pathogenesis as malaria.

FcγRIIIb polymorphism was found not to be significantly associated with any ethnic group within both the controls and the patient group. The genotype frequencies were found to be inconsistent between the control group and the patient population within any one ethnic group, and this gives an indication that this genotype is randomly distributed and not ethnically biased.

However the FcγRIIIb-NA1/NA1 genotype was found to differ significantly (P=0.03), when the patients were compared to the control group, thus indicating an association with malaria and therefore predisposing individuals to the disease (Figure 3)`. The FcγRIIIb-NA2/NA2 on the other hand seems to offer protection against all

forms of malaria, as it significantly decreased in the case of all the different disease categories. Due to the differences in the binding capabilities of the neutrophil antigens receptors 1 and 2, in which the NA1 binds IgG_1 and IgG_3 more efficiently than NA2, it was expected that the NA2 in the homozygous state would be over represented in the patients than in the healthy controls. On the contrary, it was rather the NA1/NA1 genotype, which was more represented (40.9%) among the malaria patients than the NA2/NA2 genotype (8.7%). Supposing the individuals studied were non-immune, the predominant antibody produced will be IgG_2; and since the neutrophil antigen receptors do not bind this antibody, then a person's susceptibility to severe malaria or otherwise will not be subject to his FcγRIIIb genotype. There may therefore be other properties of the FcγRIIIb-NA2/NA2 genotype which help to protect individuals against malaria.

There was significant difference found between the patients and controls possessing the heterozygous FcγRIIIb-NA1/NA2 ($P=0.001$). The results showed these heterozygotes to be intermediate between the two homozygote genotypes. This also demonstrates the protective effect of the NA2 gene, in that its presence reduces the protective effect of the NA1.

The FcγRIIIb polymorphism is thought not to play any role in an individual's predisposition to bacterial infections (Rascu *et al.*, 1997; Bredius *et al.*, 1994). The reasoning is because IgG_2 is the main antibody that fights against bacterial infections and neutrophil antigen receptors do not bind IgG_2. However in the case of malaria, there is the interplay of IgG_1, IgG_2, and IgG_3, and since these neutrophil antigens bind IgG_1 and IgG_3, the difference in their binding capabilities is likely to ensure that they influence an individual's susceptibility to malaria.

It is worthy to note that the Null genotype, where the individual possesses neither of these neutrophil antigens was more enhanced in the patient population (26.4%) than among the healthy controls (6.7%) and was found to be significantly associated (P=0.02) with malaria. This is to be expected because the absence of these neutrophil antigens will imply that phagocytosis of IgG_1 and IgG_3-opsonized parasitized erythrocytes in a malaria patient may be inefficient. The lack of these genes therefore may lead to a delayed clearance of these parasitized erythrocytes and hence an upsurge in the disease condition.

Apart from Enenkel *et al.* (1991) and Bredius *et al.* (1994) who found some Caucasians lacking the two neutrophil antigen receptors, not much is known about this condition and its relationship to any disease susceptibility. In another report by Schie and Wilson, (1997), in which the study groups were mainly Orientals and Caucasians, there was no reported incidence of the Null condition. The relative high incidence of the null condition seen in this study suggests that the condition may be probably more prevalent among Africans than their Oriental and Caucasian counterparts.

4.2 Conclusion

In conclusion, there was no significant association between the genotypes of the IgG receptors, FcγRIIa and FcγRIIIb and ethnicity, and therefore the distribution of these genetic polymorphisms was not ethnically biased in the study population.

The heterozygous FcγRIIa-H/R131 was found to be the dominant genotype of the IgG receptor FcγRIIa among the study group and may probably be the dominant genotype within the overall Ghanaian population.

A significant association was found between FcγRIIa and the incidence of severe clinical malaria. FcγRIIa-H/H131 seems to offer protection against severe malaria and cerebral malaria in the homozygous state. In the heterozygous state as H/R131, the H allele offers protection against severe anaemia but not against cerebral malaria.

The homozygous FcγRIIa-R/R131, on the other hand, may be a predisposing factor for severe anaemia and cerebral malaria. In the heterozygous state its disadvantageous effect is not hampered by the presence of the H allele in cerebral malaria.

The genotypes of the IgG receptor FcγRIIIb polymorphism were randomly and evenly distributed among the study groups. There was no prevalence of one over the others. However the null genotype was appreciably represented within the study group.

The FcγRIIIb polymorphism was also found to be significantly associated with malaria. The homozygous NA1/NA1 was found from the study to be a heritable risk factor predisposing an individual to all forms of malaria.

The NA2/NA2 on the other hand appears to offer protection against all forms of malaria. Even in the heterozygote state, its protective effect is still seen in the case of

severe anaemia and cerebral malaria. The Null genotype is also suspected to be a predisposing factor in all forms of malaria.

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APPENDICES

APPENDIX I

PREPARATION OF SOLUTIONS

The following standard solutions were prepared using sterile double distilled water (sddw). Where appropriate, the solutions were autoclaved at 121lb/sq in. for 15 minutes in an Eyela autoclave (Rikikkaki, Tokyo).

DNA Extraction

0.5% saponin

0.5g of saponin was dissolved in 100ml of freshly prepared1X Phosphate Buffered Saline (PBS)

1X Phosphate Buffered Saline

8g of NaCl, 1.44g of Na_2HPO_4 and 0.24g of KH_2PO_4 were dissolved in 800ml of sterile double distilled water and the pH adjusted to 7.4 with concentrated HCL, and volume was made to 1000ml with distilled water and the solution was autoclaved.

20% Chelex

20g of Chelex was dissolved in 100ml of distilled water and autoclaved.

5mM Sodium Phosphate

0.71g of Na_2HPO_4 was dissolved in 1000 ml of sddw and stored at - 20°C.

EtBr (10mg/ml)

1g Ethidium Bromide was completely dissolved in 100ml sddw and stored in the dark at 4°C..

Solutions for Electrophoresis

10X TAE buffer

242g Tris base, 57.1ml glacial acetic acid, 100ml 0.5M EDTA, pH adjusted to 7.7 (with glacial acetic acid) and the volume made up to 1000ml with sddw.

0.5M EDTA (pH 8.0)

186g of EDTA, dissolved in 800ml sddw, pH adjusted with NaOH pellets and volume made up to 1000ml and stored at room temperature.

Gel Loading Solutions

6X Bromophenol Blue

0.25% bromophenol blue was added to 40% sucrose in water and stored at 4°C.

5X Orange G

20% (w/v) Ficoll, 25mM EDTA, 2.5(w/v) Orange G were mixed and stored at room temperature.

APPENDIX II

SEVERE MALARIA STUDY QUESTIONAIRE

Recruit and case record form

ID number……..

Date of admission……..

Name……

Ethnic origin……

(Hausa, Fafra, Dagomba=1, Ewe=2, Ga –Adangme=3, Akan=4

Other=5 Specify----------)

Sex (1=M, 2=F)……..

Age (Last half year passed)……..

Weight (in kgs) ……..

Height (in cms)……

Referral on the basis of a lab report positive for malaria parasites (1=Yes, 2=No)…….

History of a febrile illness in the preceding 2 weeks (1=Yes, 2=No)……..

Duration of symptoms before presentation……..

Temperature (At time of blood collection)……..

Total coma score (0-5)……..

Motor Response (0-2)……..

Verbal Response (0-2)……

Eye Movement (0-1) ……

Duration of coma (0= no coma, 1=0-60mins, 2=60+ mins)……

Observed convulsions (1=Yes, 2=No)……..

Respiratory Distress (1=Yes, 2=No)……

Observed coca cola urine (1=Yes, 2=No)

Sickling status (1=positive, 2=negative)……

Parasite density (per microlitres)

Haemoglobin (Hb)……

BM-Stix (in mmol/L)……..

Exclusion criteria

Presence of Neurological disease history (1= Yes, 2=No)

Recent severe head trauma (1=Yes, 2=No)

Other causes of coma (1=Yes, 2=No)

Recent severe bleeding (1=Yes, 2=No)

Other causes of anaemia (1=Yes, 2=No)

Obvious clinical evidence of bacterial infection (1= Yes, 2=No)

Molecular biology analysis

FcγRIIa genotype H..... R.....

FcγRIIIb genotype NA1..... NA2.....

APPENDIX III

A. Patient demographics and PCR results

Code	Tribe	Age	Sex	Parasite Count	Hb	Temp	Blantyr Scale	Coma Time	Resp Dist	Cola Urine	Glucoses	Sickling	FcIIa	FcIIIb
002	Ga	4.	F	23	5.60	37.0	5	0	NO	NO	5.2	Y	H/R	NULL
003	Akan	3.	F	5963	11.20	38	5	0	NO	NO	5.9	N	R/R	N1/N2
005	Akan	9.	M	43	11.40	37.5	5	0	YES	NO	5.3	N	R/R	N1/N2
007	Akan	1.	M	244745	5.90	38.2	5	0	NO	NO	3.0	N	H/H	N1/N2
008	Ewe	2.	M	1471175	7.00	36.4	5	0	NO	NO	5.7	N	H/H	N1/N1
009	North	1.	M	434	4.30	37.0	5	0	NO	NO	5.2	N	H/R	NULL
016	Ewe	3.	M	401056	8.70	39.0	5	0	YES	YES	4.6	N	H/R	N2/N2
074	Akan	8.	F	3656	8.40	38.0	4	0	NO	NO	5.0	N	H/R	N1/N1
045	Ga	1.	F	6600	8.70	36.5	5	0	NO	NO	5.6	N	R/R	N1/N2
046	Ga	7.	M	69020	11.50	39.5	5	0	NO	YES	7.7	N	H/H	NULL
066	Akan	2.	M	25114	3.70	38.0	5	0	NO	NO	16.5	N	R/R	N1/N2
037	Akan	1.	M	94576	9.20	38.0	5	0	NO	NO	5.3	N	H/R	NULL
056	Ewe	6.	M	232170	8.30	38.0	5	0	NO	YES	5.4	N	R/R	N1/N1
087	Akan	2.	M	1213	9.50	39.0	5	0	NO	NO	7.4	N	R/R	N1/N1
057	Akan	3.	M	89856	8.60	38.0	5	0	NO	NO	9.2	N	R/R	N1/N1
033	Akan	1.	F	35308	4.40	37.5	5	0	NO	NO	5.4	N	R/R	NULL
034	Ga	4.	M	138704	4.00	39.4	0	0	NO	NO	7.2	N	R/R	N1/N1
193	Ga	3.	F	196854	5.00	37.5	2	0	NO	YES	5.1	N	H/H	N2/N2
171	Akan	5.	M	58899	8.00	39.0	2	0	NO	NO	7.0	N	H/R	N1/N2
130	North	5.	M	193874	8.30	37.0	1	1	YES	NO	0.2	N	H/R	NULL
135	North	5.	M	327105	8.70	37.0	2	2	NO	NO	8.7	N	H/R	N1/N1
144	Akan	2.	M	35250	5.20	36.8	2	2	YES	YES	11.6	N	H/R	N1/N1

195	Ga	4.	M	123997	4.20	37.5	2	2	NO	YES	11.3	N	H/R	N2/N2
172	Akan	130	M	7191	12.40	37.5	5	0	NO	NO	5.1	N	H/H	N1/N2
190	Ewe	3.	F	291890	3.90	39.4	5	0	YES	NO	5.8	N	H/H	NULL
183	Akan	10	M	126720	14.10	37.3	5	0	NO	NO	11.0	N	H/R	N1/N1
169	North	4.	M	599010	6.60	36.0	5	0	NO	YES	5.6	N	H/R	NULL
163	Akan	3.	M	233011	8.40	36.5	5	0	NO	NO	5.3	N	H/H	N1/N1
160	Akan	7.	M	190404	11.50	38.6	5	0	NO	NO	4.2	N	H/R	N1/N2
168	North	6.	F	87395	8.60	37.0	5	0	NO	NO	5.1	N	H/R	N1/N2
188	Akan	2.	M	43	2.90	36.9	5	0	YES	NO	4.1	N	H/R	NULL
146	North	10	M	16539	7.80	37.0	5	0	NO	YES	5.9	N	H/R	NULL
161	North	4.	F	43069	7.80	40.1	5	0	NO	NO	3.5	N	H/R	N1/N2
166	Ga	5.	F	7345	5.40	37.0	5	0	NO	NO	5.1	N	H/R	N1/N2
134	Ewe	2.	F	302	9.50	39.0	5	0	NO	NO	3.3	N	H/R	NULL
192	Akan	7.	M	70	5.30	38.8	5	0	NO	YES	9.4	N	H/H	NULL
185	North	6.	F	43289	4.40	36.0	4	2	YES	YES	5.6	N	R/R	N1/N1
157	Ga	4.	M	3595	6.70	38.5	5	0	NO	YES	4.9	N	R/R	N1/N2
132	Akan	4.	F	91379	7.10	39.4	5	0	NO	NO	5.8	N	R/R	N1/N1
127	Akan	1.	M	6586	3.90	39.4	5	0	YES	NO	4.8	N	R/R	NULL
140	Ga	1.	F	237451	5.50	38.5	5	0	NO	YES	7.8	N	R/R	NULL
150	Akan	6.	F	26726	8.20	37.2	5	0	NO	NO	6.2	N	H/R	NULL
156	Akan	1.	F	140	6.00	36.5	5	0	YES	NO	6.1	N	H/R	N1/N2
143	North	1.	F	2021	6.30	37.5	5	0	NO	YES	5.2	N	H/H	NULL
141	Akan	5.	M	5773	4.20	39.0	5	0	NO	YES	6.0	1	H/R	N1/N1
181	Ewe	3.	M	50862	5.40	37.0	5	0	NO	YES	4.5	N	H/R	N1/N1
177	Ewe	1.	F	392	6.00	38.0	4	0	YES	NO	9.0	N	H/R	N1/N1
145	Akan	3.	M	131455	5.10	37.0	5	0	NO	YES	6.7	N	H/R	NULL
186	Akan	4.	F	418980	4.90	36.5	5	0	YES	NO	7.0	N	R/R	N1/N1
196	Ewe	1.	M	108367	3.60	36.8	5	0	NO	YES	5.8	N	H/H	N2/N2
194	Akan	2.	M	1462	4.30	38.2	5	0	NO	NO	3.8	N	R/R	N1/N1
199	Akan	1.	F	210680	5.00	37.0	5	0	NO	NO	6.8	N	R/R	N1/N1

125	North	4.	M	100775	4.50	37.5	5	0	NO	NO	7.8	N	R/R	N1/N1
153	Akan	2.	F	21672	5.00	37.8	5	0	NO	YES	5.6	N	R/R	NULL
255	Ga	2.	M	11655	9.10	38.0	2	0	NO	NO	7.9	N	H/R	N1/N1
261	Ewe	11	M	8881	8.50	37.0	4	2	NO	NO	5.1	N	H/R	N2/N2
232	Ewe	11	M	142197	6.60	38.5	2	1	NO	NO	6.0	N	H/R	N1/N1
297	Akan	4.	F	19521	4.10	39.0	4	1	NO	YES	6.4	N	H/R	N2/N2
271	Akan	6.	F	258463	7.80	38.4	5	0	NO	NO	7.1	N	H/H	N2/N2
218	Akan	8.	M	34	10.20	37.5	2	2	NO	NO	10.8	N	H/R	N2/N2
284	Ewe	3.	M	27368	5.30	37.5	2	2	YES	YES	11.3	N	H/R	N1/N1
276	Ewe	12	M	60732	10.90	37.7	5	0	NO	NO	6.5	N	H/R	N1/N1
272	Akan	7.	F	218920	10.60	39.0	5	0	NO	NO	9.5	N	H/R	N1/N1
267	Ga	9.	M	19030	9.50	39.0	5	0	NO	YES	6.9	N	H/R	N1/N1
221	Ewe	1.	F	38922	10.00	37.0	5	0	NO	NO	5.7	N	H/R	N1/N1
238	North	1.	M	332000	6.20	40.0	5	0	NO	NO	5.9	N	H/R	N1/N1
241	Ga	4.	M	24568	7.80	38.7	3	2	NO	YES	6.0	N	H/R	N1/N1
213	Akan	2.	M	16	10.40	36.8	5	0	NO	NO	6.3	N	H/R	NULL
291	Ewe	2.	M	12	4.10	36.5	5	0	NO	NO	4.9	N	H/R	NULL
288	Akan	11	F	408	9.10	38.0	5	0	NO	NO	5.3	N	H/R	N2/N2
234	North	1.	F	4535	1.90	38.0	2	0	YES	NO	4.6	N	H/R	N2/N2
295	Ewe	1.	M	34	4.90	38.0	5	0	NO	NO	5.7	N	H/R	NULL
207	Ga	2.	F	2040	3.40	38.0	5	0	YES	NO	5.0	N	H/R	N1/N1
266	North	7.	M	48438	6.80	37.2	5	0	NO	YES	6.8	N	H/H	N1/N2
231	North	3.	F	93555	5.90	39.0	2	1	NO	YES	5.7	N	H/R	N1/N2
224	North	2.	M	2330	6.40	37.2	5	0	NO	NO	5.0	N	H/R	NULL
223	North	2.	F	72680	.60	38.7	3	2	NO	NO	5.6	N	R/R	N1/N1
214	Akan	1.	M	4280	4.10	38.0	5	0	YES	YES	6.5	N	H/R	N2/N2
230	North	12	F	131708	7.00	37.0	5	0	NO	NO	7.5	N	H/R	N1/N1
246	North	1.	M	801	3.40	36.0	5	0	YES	NO	4.7	N	H/R	NULL
243	Akan	1.	F	98745	7.00	37.0	5	0	NO	NO	7.1	N	H/R	NULL
217	Akan	5.	M	32	5.20	37.0	5	0	NO	YES	6.3	N	R/R	NULL

294	Ga	8.	M	15	4.90	38.0	5	0	NO	YES	5.6	N	H/R	N1/N1
239	North	2.	M	3427	8.00	39.2	5	0	NO	NO	4.4	N	H/R	NULL
233	North	1.	F	12528	6.00	39.2	4	0	YES	NO	6.4	N	H/R	N1/N1
292	Akan	2.	F	30	3.60	38.0	5	0	NO	YES	5.9	N	H/R	N1/N1
293	Akan	1.	M	1740	4.50	36.0	5	0	NO	NO	6.3	N	H/R	NULL
287	Akan	8.	F	2555	8.10	36.0	5	0	NO	NO	5.6	N	H/R	N1/N1
252	Ewe	6.	F	19	4.40	39.4	2	2	NO	YES	7.9	N	H/R	N1/N1
250	North	4.	M	25	4.60	36.0	5	0	NO	NO	6.6	N	H/R	N1/N1
275	Akan	6.	M	15997	9.50	37.5	5	0	NO	NO	6.2	N	H/R	N1/N2
274	Ga	1.	M	31	11.10	38.0	5	0	NO	NO	5.4	N	H/R	N1/N2
248	North	2.	F	299932	7.80	38.0	5	0	NO	NO	8.1	N	H/R	NULL
253	Akan	1.	F	110208	3.20	39.0	5	0	NO	NO	4.6	N	R/R	NULL
280	Ga	1.	F	55505	4.70	37.2	5	0	YES	NO	6.4	N	R/R	N1/N1
249	Ga	9.	F	36704	.60	37.8	5	0	YES	NO	7.9	N	R/R	NULL
258	North	1.	M	150700	4.50	36.9	5	0	NO	YES	5.7	N	R/R	N1/N1
205	Ga	2.	M	13872	2.00	36.8	5	0	NO	NO	4.9	N	H/H	N1/N2
226	Akan	4.	M	83996	3.60	37.5	5	0	NO	NO	5.0	N	R/R	N1/N1
245	Ewe	2.	F	48608	4.10	36.0	5	0	NO	YES	1.6	N	R/R	NULL
283	Akan	3.	M	239169	6.50	38.0	3	2	NO	YES	5.5	N	H/R	N1/N1
269	Akan	1.	M	20	.80	37.4	5	0	NO	NO	5.9	Y	H/R	N1/N1
266	North	7.	M	48438	6.80	37.2	5	0	NO	1	6.8	N	R/R	N1/N1
265	Akan	3.	F	22	10.30	38.5	5	0	NO	NO	5.9	N	R/R	N1/N1
256	Akan	1.	F	174603	5.70	38.0	5	0	NO	NO	5.0	N	R/R	NULL
259	Akan	5.	M	121004	5.90	37.0	5	0	NO	NO	5.7	N	H/R	N1/N1
260	Ewe	4.	F	33	10.10	39.0	2	1	NO	YES	6.5	N	R/R	NULL
263	Akan	4.	M	197	11.60	37.0	2	0	YES	NO	8.1	N	R/R	N1/N1
204	North	1.	F	39711	3.70	37.0	5	0	NO	NO	6.1	Y	R/R	NULL
373	Akan	3.	F	113400	7.60	36.5	2	2	NO	NO	5.8	N	R/R	N1/N1
370	North	9.	M	43043	2.00	36.4	0	2	NO	NO	11.3	N	H/R	N1/N1
353	North	12	F	347645	1.30	38.0	1	2	NO	NO	7.2	N	R/R	N1/N1

338	North	5.	M	258439	8.00	39.0	2	2	NO	YES	8.3	N	H/R	N1/N1
307	Ga	2.	F	145850	5.10	37.0	2	2	YES	YES	5.7	N	H/R	N1/N2
319	Ewe	7.	M	376720	5.10	39.5	3	0	NO	NO	6.6	N	H/R	N1/N1
337	Akan	2.	F	115650	8.10	37.5	5	0	NO	NO	7.3	N	H/R	N1/N1
333	Ewe	2.	M	63212	8.30	37.0	5	0	NO	NO	4.5	N	H/R	N1/N1
326	North	9.	F	49392	8.70	37.0	5	0	NO	NO	4.7	N	H/H	N1/N1
320	North	3.	M	276345	8.90	39.4	5	0	NO	NO	5.6	N	H/R	N1/N1
380	Akan	1.	F	31824	8.30	37.0	5	0	NO	NO	4.4	N	H/H	N1/N2
358	North	4.	M	48825	8.80	36.0	5	0	NO	NO	9.5	N	H/R	N1/N1
336	Akan	1.	F	165322	3.40	37.2	5	0	NO	NO	6.7	N	H/R	N1/N2
339	Ewe	9.	F	109630	6.20	37.0	3	2	NO	YES	8.1	N	R/R	N2/N2
331	Akan	5.	M	73980	5.70	37.0	5	0	NO	NO	5.9	N	R/R	N1/N2
301	Akan	2.	M	3915	4.00	36.2	5	0	NO	NO	6.2	Y	H/R	NULL
300	Akan	2.	M	190156	5.40	38.5	5	0	NO	YES	6.6	N	R/R	NULL
359	Ga	4.	F	282296	5.80	39.9	2	2	YES	YES	3.2	N	H/R	NULL
322	Ga	4.	M	101600	7.60	37.5	5	0	NO	NO	6.6	N	R/R	NULL
356	Ga	1.	F	171234	4.50	38.0	3	1	YES	YES	9.9	N	H/R	NULL
350	North	3.	F	729	5.20	39.9	2	0	NO	YES	16.1	N	H/R	NULL
341	Akan	2.	M	122724	5.20	36.5	5	0	NO	NO	4.7	N	H/H	NULL
352	North	5.	F	53210	8.50	38.0	3	2	NO	YES	12.6	N	R/R	NULL
309	North	4.	M	2070	6.10	37.0	5	0	NO	YES	8.6	N	H/R	N1/N2
366	Ewe	2.	F	779	4.90	37.0	5	0	NO	YES	5.8	N	R/R	NULL
376	North	1.	M	1776	3.80	37.0	5	0	NO	NO	3.9	N	R/R	N1/N1
371	Ewe	8.	M	29403	4.30	37.5	5	0	NO	YES	4.6	Y	H/H	N1/N2
321	Akan	1.	M	18336	4.90	36.9	5	0	NO	NO	6.1	N	H/H	N1/N2
324	Ga	2.	M	147672	3.70	37.5	5	0	NO	NO	4.3	N	R/R	N1/N1
345	North	1.	F	18954	3.40	36.8	5	0	NO	YES	4.4	N	R/R	NULL
374	Akan	4.	M	10020	3.50	37.0	5	0	NO	NO	4.8	N	R/R	N1/N1
318	Ga	1.	M	21463	3.20	37.0	5	0	NO	NO	6.0	N	R/R	N1/N1
314	Akan	1.	F	87006	2.30	37.0	2	2	YES	NO	3.6	N	R/R	NULL

303	Ga	2.	F	387113	5.00	38.0	5	0	NO	YES	7.5	N	R/R	N1/N1
317	Akan	1.	M	36916	3.30	36.0	5	0	NO	NO	5.7	N	H/H	N2/N2
311	Akan	2.	M	19065	3.80	36.0	5	0	NO	NO	5.4	N	R/R	N1/N1
329	North	2.	M	27	2.50	36.0	5	0	NO	NO	2.6	N	R/R	NULL
327	North	5.	M	98425	4.70	37.0	3	2	NO	YES	6.1	N	R/R	N1/N1
357	North	8.	M	136940	10.80	38.4	1	2	YES	NO	11.9	N	H/H	N1/N2
383	Akan	3.	M	332424	4.20	37.8	2	2	NO	YES	5.6	N	H/H	N1/N1
385	Akan	7.	M	24450	9.80	36.5	5	0	NO	YES	4.6	N	H/R	N1/N1
389	Akan	4.	F	20352	5.50	38.0	2	2	NO	NO	2.4	N	H/R	N1/N1
393	Ga	2.	F	475145	8.10	38.4	2	0	YES	NO	1.2	N	R/R	NULL
391	North	11	M	148	8.70	37.3	5	0	NO	NO	6.1	N	H/R	N1/N2
392	Ewe	6.	F	297	11.50	38.5	5	0	NO	NO	4.8	N	H/R	N1/N2
394	Akan	6.	M	2736	8.00	36.2	5	0	NO	YES	5.0	N	H/R	N1/N1
395	Akan	2.	M	4901	9.00	38.5	5	0	NO	YES	4.4	N	H/R	NULL
396	Akan	3.	M	3255	7.50	36.5	5	0	NO	NO	2.8	N	H/R	N1/N1
398	Akan	3.	F	303776	7.40	38.8	5	0	NO	NO	9.8	N	R/R	N1/N1
399	Akan	3.	F	78942	4.70	38.8	5	0	YES	YES	5.6	N	R/R	N1/N1
384	North	2.	F	618	8.20	36.2	3	2	YES	NO	8.4	N	H/R	N1/N1
386	North	1.	M	342	8.70	38.5	5	0	NO	NO	5.8	N	H/R	N2/N2
387	North	4.	M	160	4.80	36.6	5	0	NO	NO	6.3	N	H/R	N1/N1
402	Ga	2.	M	4912	3.00	39.0	5	0	NO	NO	5.6	N	R/R	NULL
403	Ewe	1.	M	382000	8.20	36.0	5	0	NO	NO	4.2	N	H/H	NULL
405	Ewe	1.	M	5232	4.60	37.6	5	0	NO	NO	7.8	N	H/R	N1/N1
400	Ga	3.	M	41572	3.60	36.4	5	0	YES	NO	3.2	N	R/R	N1/N1
401	Ewe	3.	M	24174	3.30	39.0	5	0	NO	YES	5.0	N	R/R	NULL
408	North	4.	F	78204	4.00	38.2	5	0	NO	YES	5.1	N	R/R	N1/N1
409	Ga	2.	F	126132	4.90	37.0	5	0	NO	NO	5.6	N	R/R	N1/N1
411	Ewe	2.	F	81780	4.90	37.5	5	0	NO	NO	4.1	N	R/R	N1/N1
412	Akan	10	M	18924	4.70	37.2	5	0	YES	NO	5.1	N	H/R	N1/N1
416	North	3.	F	130739	3.80	38.5	5	0	YES	NO	3.7	N	R/R	N1/N1

418	Akan	5.	M	78864	3.70	38.5	5	0	YES	NO	7.2	N	H/R	N1/N1
419	Ga	5.	M	17874	5.00	38.5	5	0	NO	NO	4.8	N	H/R	N1/N2
435	Akan	2.	F	12272	3.90	37.8	5	0	NO	NO	5.6	N	R/R	N1/N1
436	Akan	1.0	F	9845	8.90	36.5	5	0	NO	NO	8.4	N	H/R	N1/N1
437	Akan	5.	F	29963	8.60	39.2	5	0	NO	NO	5.1	N	H/R	N1/N1
444	Akan	7.	F	74556	9.60	36.5	2	2	YES	NO	6.6	N	H/R	N1/N1
446	Akan	9.	F	55699	4.60	39.0	3	2	NO	NO	7.2	N	H/R	N1/N1
451	Ewe	1.	M	27984	4.80	36.5	5	0	NO	YES	5.8	N	R/R	N1/N1
453	North	2.	M	11658	3.10	36.5	5	0	NO	NO	3.9	N	H/R	N1/N1
454	Ga	5.	F	24816	3.70	37.0	5	0	NO	NO	11.5	N	H/R	N1/N2
456	Akan	3.	F	188928	5.40	39.0	2	0	NO	NO	7.9	N	H/R	N1/N2
475	North	4.	F	132000	4.40	38.4	5	0	YES	NO	5.0	N	H/R	N1/N2
476	Akan	1.	F	253760	7.20	38.0	3	0	YES	YES	4.7	N	H/R	N1/N2
413	Akan	3.	F	178500	5.40	38.0	5	0	NO	NO	6.2	N	H/R	N2/N2
421	Akan	2.	M	351	6.30	37.8	5	0	NO	NO	5.5	N	R/R	NULL
425	Akan	5.	F	2320	6.90	37.2	5	0	NO	NO	4.6	N	R/R	NULL
426	Ga	1.	M	1658	7.00	37.0	5	0	NO	NO	4.0	N	H/H	NULL
427	Ewe	2.	M	353101	6.50	36.9	5	0	NO	YES	1.3	N	H/R	N1/N2
428	Ga	5.	M	366933	10.20	37.6	3	2	NO	YES	7.8	N	H/R	NULL
440	North	2.	M	23400	5.40	38.0	5	0	NO	NO	5.6	N	R/R	NULL
442	Akan	4.	M	88808	1.00	38.4	5	0	NO	NO	2.2	N	R/R	NULL
445	North	2.	M	84800	.50	39.0	5	0	NO	YES	15.1	N	R/R	N1/N1
447	Ga	9.	M	11	3.60	36.8	2	2	NO	YES	6.1	N	R/R	NULL
448	North	1.	F	972876	3.10	36.5	5	0	NO	NO	2.0	N	H/R	NULL
452	North	2.	F	528	5.80	36.5	5	0	NO	NO	4.9	N	R/R	NULL
457	Akan	5.	F	42920	7.40	38.8	5	0	NO	NO	8.0	N	R/R	N1/N1
461	Akan	1.	M	9792	5.60	36.2	5	0	NO	YES	5.1	N	R/R	N2/N2
465	Akan	3.	F	425	6.60	39.2	5	0	NO	NO	6.2	N	H/H	N1/N1
467	North	2.	M	108575	5.50	38.2	5	0	NO	NO	9.9	N	H/H	NULL
469	Ewe	5.	M	392520	7.50	36.0	3	2	NO	NO	2.7	N	R/R	N1/N1

471	North	1.	F	819	9.00	39.0	5	0	NO	NO	3.2	Y	H/R	N2/N2
474	North	3.	M	2772889	.40	37.0	1	2	YES	NO	9.5	N	H/R	N1/N2
480	Akan	12	F	1435	6.40	37.0	5	0	NO	YES	5.4	Y	H/R	N1/N1
036	Akan	5.	F	20770	8.70	40.0	5	0	NO	YES	5.3	N	R/R	N1/N1
044	North	3.	M	667440	7.30	38.8	5	0	NO	YES	5.3	N	H/R	N1/N2
149	Akan	3.	M	1955	12.20	39.4	5	0	NO	NO	5.5	N	H/R	N1/N2
170	Akan	1.	F	148372	4.80	37.5	5	0	NO	NO	7.2	Y	H/R	N1/N2
178	Ga	4.	F	144606	7.30	38.3	2	1	YES	YES	19.1	N	H/R	N1/N2
208	Ga	2.	F	1331	7.00	36.8	5	0	NO	NO	4.2	N	H/R	N1/N2
209	Akan	3.	M	103296	6.30	37.5	3	2	NO	YES	4.0	N	R/R	N1/N1
210	North	1.	M	45314	5.80	39.4	5	0	YES	YES	6.1	N	H/R	NULL
211	North	7.	M	1065	7.10	37.5	5	0	NO	YES	9.5	N	H/R	N1/N2
216	Akan	6.	M	35075	6.60	37.0	5	0	NO	NO	4.7	N	H/R	N1/N2
222	Ewe	3.	M	55616	4.70	37.5	5	0	NO	YES	8.0	N	H/H	N1/N2
240	Ga	10	F	62730	6.50	37.0	5	0	NO	YES	4.8	N	H/R	N1/N1
242	Akan	10	M	228	6.10	38.4	5	0	NO	YES	6.1	N	H/R	N1/N1
254	Ewe	2.	F	2772	4.90	38.4	5	0	NO	NO	5.4	N	H/R	N1/N1
257	Ewe	1.	M	98600	4.30	39.0	5	0	YES	NO	1.3	N	H/R	N2/N2
262	Ewe	3.	M	153468	2.10	36.5	5	0	NO	1	6.1	N	H/R	N1/N2
264	North	2.	F	6129	3.50	37.4	5	0	NO	NO	4.9	N	R/R	NULL
270	Akan	6.	M	7802	9.30	37.5	5	0	NO	YES	5.4	N	H/H	N1/N2
277	Ga	2.	M	12	8.00	38.0	2	2	NO	NO	5.8	N	H/R	N1/N2
278	Akan	1.	F	1700	4.30	36.0	5	0	NO	NO	9.2	N	H/R	N1/N2
279	Ga	6.	M	270427	6.60	36.0	5	0	NO	NO	6.5	N	H/R	N1/N1
281	Ga	8.	F	540	4.50	36.6	5	0	NO	NO	8.4	N	H/R	N1/N1
282	Akan	2.	F	39816	7.90	36.0	5	0	NO	NO	5.8	N	H/R	NULL
315	Ewe	3.	F	13518	7.90	35.0	5	0	NO	NO	9.3	N	H/R	N1/N2
323	Ewe	1.	F	662	2.80	37.0	5	0	NO	NO	5.8	N	H/R	N1/N2
325	North	4.	M	24	12.50	37.0	5	0	NO	NO	7.7	N	H/R	NULL
328	Akan	3.	M	182	2.50	37.0	5	0	NO	YES	6.7	N	H/R	N2/N2

330	North	1.	M	13398	5.10	38.0	5	0	NO	YES	6.9	N	H/R	N1/N2
334	Ewe	7.	M	176015	9.40	37.0	5	0	NO	NO	5.7	N	H/R	N2/N2
335	North	5.	M	2093	3.30	37.0	2	2	NO	NO	6.7	N	R/R	N1/N2
344	Ga	8.	M	17596	6.10	36.5	5	0	NO	YES	7.6	N	H/R	NULL
348	Akan	2.	F	440	8.10	37.0	5	0	NO	NO	7.7	N	H/H	N2/N2
360	Akan	5.	M	44604	1.20	38.0	5	0	NO	YES	4.2	N	H/R	N1/N1
369	Akan	3.	M	26137	9.00	37.8	5	0	NO	NO	7.0	N	H/R	N1/N2
377	Ewe	2.	F	21	5.10	37.0	5	0	NO	NO	4.3	N	H/R	N1/N1
378	Akan	1.	F	140	3.80	36.8	5	0	NO	YES	5.0	N	R/R	N2/N2
379	Akan	4.	M	86	5.80	36.0	5	0	NO	NO	4.9	N	H/R	N1/N2
381	Akan	5.	F	3015	9.10	37.8	4	0	NO	YES	5.0	N	R/R	N1/N2
388	Akan	2.	M	17721	6.40	37.5	5	0	NO	NO	6.6	N	H/R	N1/N2
415	Akan	11	M	47544	5.60	36.6	5	0	NO	YES	8.1	N	H/R	N1/N2
420	North	3.	M	180183	6.40	36.2	5	0	NO	NO	4.3	N	H/R	N1/N2
423	Akan	3.	F	90522	8.40	37.0	5	0	NO	NO	6.7	N	H/R	N1/N1
434	Ewe	4.	M	23496	5.30	39.9	5	0	NO	NO	5.1	N	H/R	N1/N1
455	Akan	2.	M	68700	9.10	38.0	5	0	NO	NO	4.2	N	H/R	NULL
462	North	6.	M	4725	4.40	39.0	5	0	NO	YES	4.9	N	H/R	N1/N2
464	North	12	F	112736	7.00	38.5	5	0	NO	NO	3.9	N	H/R	N1/N1
468	Akan	6.	M	100100	5.20	37.2	5	0	NO	NO	10.9	N	H/R	N1/N2
472	Akan	6.	M	5	11.30	36.5	5	0	NO	YES	3.7	N	H/R	N1/N2
478	Akan	2.	F	4722	6.90	37.0	5	0	YES	NO	4.9	N	H/R	N1/N2

B. Control group demographics and PCR results

code	tribe	sex	age	temp	Wt/kg	Fcγ IIa	FcγIIIb
DD002	Ga	M	4.	36.60	18.00	H/R	N2/N2
DD004	Ga	F	7.	36.10	22.00	H/R	N1/N2
DD005	Ga	M	9.	36.30	27.00	R/R	N2/N2
DD014	Ga	F	9.	36.50	25.00	H/R	N1/N2
DD015	Ga	F	11	7.70	60.00	H/R	N2/N2
DD016	Ga	M	2.	36.00	12.00	H/R	N2/N2
DD017	North	F	7.	36.90	17.50	H/H	N1/N2
DD018	Ga	F	12	36.60	31.50	R/R	N1/N1
DD019	Ga	M	13	36.00	31.00	H/R	N1/N2
DD021	Ga	F	6.	36.60	15.00	H/H	N2/N2
DD023	Ga	F	6.	36.30	14.00	H/R	N1/N2
DD026	North	M	7.	36.50	30.00	H/R	N2/N2
DD027	Ga	F	8.	36.20	22.00	H/R	N2/N2
DD028	Ga	F	1	36.20	28.20	H/R	N2/N2
DD032	Ga	F	10	36.60	25.00	H/R	N1/N2
DD035	Ga	F	7.	36.10	18.50	H/R	N2/N2
DD039	Ga	F	5.	36.40	17.50	R/R	N1/N1
DD041	Akan	M	7.	36.80	16.50	H/R	N2/N2
DD042	Akan	M	9.	36.50	21.00	H/R	N1/N1
DD047	Ga	F	.	36.60	26.50	H/R	N1/N2
DD051	Akan	M	2.	36.10	13.20	H/H	N1/N1
DD059	North	F	7.	37.00	24.00	R/R	N1/N2
DD063	North	F	13	37.00	40.00	R/R	N2/N2
DD065	North	F	8.	36.20	24.50	H/H	N2/N2
DD076	Ga	M	9.00	36.80	24.00	R/R	N1/N2
DD078	Ga	F	10.	37.00	25.00	H/H	N1/N2
DD079	Ga	F	9.	36.00	27.00	H/H	N1/N2
DD081	Akan	M	5.	36.80	19.10	H/R	N1/N1
DD082	Akan	F	5.	37.20	14.00	R/R	N1/N1
DD083	Ga	M	3.	36.50	16.00	H/R	N1/N1
DD084	North	M	12	37.10	28.00	H/R	N2/N2
DD085	North	M	9.	37.20	22.00	H/R	N1N2
DD086	Akan	M	3.	36.30	12.00	H/R	N1N2
DD087	Ga	M	6.	36.70	17.00	H/H	N2/N2
DD088	North	F	10	37.20	27.00	H/R	N1/N1
DD090	North	M	7.	37.00	17.00	H/R	NULL
DD103	Ewe	M	8.	37.20	25.00	H/H	N1N2
DD104	Ewe	M	12	36.80	42.00	R/R	NULL
DD105	Ewe	F	4.	37.30	13.00	H/R	N2/N2
DD108	Ewe	M	4.	37.20	11.50	H/H	N2/N2
DD109	Ewe	M	5.	36.40	15.00	R/R	N2/N2
DD110	North	M	3	36.10	29.00	R/R	N2/N2
DD125	Ewe	F	5.	36.60	13.00	H/H	N2/N2
DD128	Ewe	F	4.	36.90	15.50	H/R	N2/N2

DD130#	Ga	F	6.	37.20	19.00	R/R	N1N2
DD131	Akan	M	6.	37.40	17.00	H/R	N2/N2
DD132	Ewe	M	11	37.40	27.00	H/R	N2/N2
DD133	North	M	3.	37.20	15.00	H/R	N2/N2
DD134	Ewe	M	9.	36.40	26.00	R/R	N1N2
DD135	Ewe	M	8.	36.50	14.00	H/R	N1N2
DD138	Ga	F	10	36.50	26.50	H/R	N1N2
DD139	Ga	F	12.	37.40	26.00	H/R	N1N2
DD149	Ga	F	2.	36.60	11.50	H/R	N1N2
DD150	Akan	M	10	37.20	26.50	H/H	N1/N1
DD151	Ga	M	9.	37.00	25.00	H/R	N1/N1
DD153	Akan	M	6.	36.20	18.00	R/R	N1N2
DD154	Ewe	M	10	35.80	27.00	H/R	N1N2
DD156	Ga	M	8.	37.20	27.00	H/R	N1N2
DD159	Ewe	F	8.	36.60	18.00	R/R	N1N2
DD160	Ewe	F	4.	36.50	12.00	R/R	N1/N1
DD161	Ewe	M	10	36.20	30.00	H/H	N1N2
DD166	Ewe	F	11	36.60	35.00	H/R	N2N2
DD167	Ewe	F	19.	35.10	53.00	H/H	N1N2
DD169	Ewe	M	3.	36.90	11.00	H/H	N1N2
DD172	Ga	M	4.	37.50	14.00	H/H	N1N2
DD181	Ga	F	9.	36.00	23.00	H/R	N1N2
DD189	Ewe	F	9.	38.00	29.00	H/R	NULL
DD190	Akan	F	3.	36.40	10.50	H/R	N1N2
DD192	Ewe	M	4.	37.20	14.50	H/R	N1N2
DD193	Ewe	M	0.	37.00	20.00	R/R	NULL
DD195	Ewe	F	11.	36.90	33.00	H/R	N1N2
DD196	Ewe	F	.8	36.80	18.00	H/R	N1N2
DD197	Akan	M	8.	37.20	22.00	H/R	NULL
DD198	North	M	6.	36.90	17.00	H/R	N1N2
DD199	North	F	2.	36.10	12.00	H/R	N1N2