Assessment of co-segregated TLR4 genotypes among Nigerian children with asymptomatic and clinical malaria

Bamidele Abiodun Iwalokun1*, Senapon Olusola Iwalokun2, Bernice Enobong Udoh3, Modinat Balogun4

1Molecular Biology and Biotechnology Division, Nigerian Institute of Medical Research, Lagos, Nigeria
2Olorunda Primary Health Centre, Lagos, Nigeria
3Department of Medical Microbiology and Parasitology, Olabisi Onabanjo University, Ogun State, Nigeria
4Iru Primary Health Centre, Lagos, Nigeria

ABSTRACT

Objective: To assess the occurrence and pattern of Toll-like receptor 4 (TLR4) co-segregated genotypes among children with Plasmodium falciparum malaria in Nigeria.

Methods: In this case-control study, a total of 79 Plasmodium falciparum infected children aged 2–7 years and 105 age-matched uninfected controls of Yoruba descent in Lagos were studied. The extracted DNA samples were used for TLR4 genotyping at codons 299 (Asp>Gly) and 399 (Thr>Ile) by PCR-restriction fragment length polymorphism. Malaria infection was diagnosed by blood smear microscopy and infected children were stratified into asymptomatic, uncomplicated and severe malaria sub-groups. Malnutrition was determined by measuring the mid upper arm circumference and anemia was defined as hemoglobin < 11 g/dL.

Results: The proportions of children with acute malnutrition and severe anemia were 12.0% and 3.2%, respectively. Parasitemia and malnutrition were not correlated and four distinct patterns of TLR4 genotypes were found in the study population: Asp299Asp/Thr399Thr (90.2%), Asp299Gly/Thr399Thr (4.3%), Gly299Gly/Thr399Thr (3.8%) and Asp299Gly/Thr399Ile (1.6%). These genotypes did not differ significantly (P > 0.05) in frequency between infected and non-infected children. However, low and high occurrences of the TLR4 Asp299Asp/Thr399Thr and Asp299Gly/Thr399Thr genotypes were observed in the severe malaria subgroup.

Conclusions: This study reveals a protective role for TLR4 Asp299Gly/Thr399Ile and Asp299Asp/Thr399Thr genotypes against severe malaria in Nigerian children.

1. Introduction

Although significant improvement in malaria control/elimination efforts has resulted in the global reduction in malaria mortality by 65%, the African continent still disproportionately bears the greatest malaria burden [1]. In 2015, an estimated 306,000 deaths occurred in children below age 5 years with African children accounting for 95.4% of these deaths. Globally, the number of malaria deaths reported in 2015 was 438,000 [1]. This represented 0.2% of the 214 million clinical malaria cases [1]. However, factors responsible for a small percentage of children eliciting severe malaria remain incompletely understood. Meanwhile, molecular evidence has attributed 25% of malaria susceptibility to genetic factors [2]. Among these factors are genes associated with pathogen recognition and innate immune response. Toll-like receptor 4 (TLR4) gene on human chromosome 9 encodes TLR4 receptor, which is well known for Plasmodium glycosylphosphatidylinositol ligand
binding in humans and rodents [3,4]. The ligand binds within the leucine rich repeat of TLR4 receptor. Two polymorphisms in TLR4 at codon 299 with aspartate being replaced by glycine (Asp299Gly) and codon 399 with threonine being replaced by isoleucine (Thr399Ile) have been associated with poor malaria parasite recognition with subsequent impairment in the production of pro-inflammatory cytokines at the early onset of infection (4–6). In recent times there has been an increased interest from malaria endemic countries towards understanding the role played by TLR4 Asp299Gly and TLR4 Thr399Ile single nucleotide polymorphisms (SNPs) in malaria pathogenesis. However, a few immunogenetic studies in Africa have reported varying frequencies of these TLR4 SNPs and their contrasting contributions to malaria susceptibility [7–10]. Nigeria, with an estimated 161 million population is endemic for Plasmodium falciparum (P. falciparum) malaria. The country is among the 15 countries responsible for 88% of global malaria burden in 2015 [1]. We have previously shown that TLR4 polymorphisms due to Asp299Gly and Thr399Ile mutations influence susceptibility to clinical malaria among Yoruba children with the minor alleles 299Gly and 399Ile occurrence at 17.6% and 14.7% respectively among children with severe malaria [11]. However, information regarding the occurrence of these polymorphisms in a co-segregated state was lacking. Given the fact that local evidences exist for high occurrence of asymptomatic malaria, uncomplicated malaria and severe malaria in Nigerian children, data on the co-segregated TLR4 Asp299Gly/Thr399Ile genotypes could be helpful in strengthening an early suspicion of children that are more likely to be susceptible/protected from clinical and severe malaria in the country. In this study, we assessed the occurrence and pattern of TLR4 co-segregated genotypes in Nigerian children of Yoruba descent infected with P. falciparum in Lagos. The frequencies of these genotypes were also compared between the clinical states of malaria and with those of other malaria endemic countries of the world.

2. Materials and methods

2.1. Setting and study population

The enrollment sites for this study comprised Takwabay Health Post and Iru Primary Health Centre on Victoria Island and Massey Street Children Hospital on Lagos Island. Enrollment of children with asymptomatic and uncomplicated malaria at Takwabay in 2009 has previously been described [11]. In place of Ibeshe community used in the previous study, data from children enrolled at Iru Primary Health Centre during the Maternal and Child Health weeks of March and May, 2012 were used. This enrollment actually enabled us not only to conduct TLR4 genotyping on age and sex-matched asymptomatic malarial and uncomplicated malarial children but also on those who were not infected that were absent in the previous study. The enrollment of children with severe malaria at Massey Street Children Hospital has also been previously described [11].

On the whole, a total of 79 malaria infected children aged 2–7 years with a mean age of 3.7 years and 105 age-matched non-infected children were enrolled. Both the case and control populations were also sex-matched, comprising 57.1% and 57% males, respectively. Enrollment of children into this study was based on the willingness of the children and their caregivers to participate in the study procedures and absence of any febrile condition apart from malaria. Asymptomatic malaria was defined as parasitemia without fever in the previous 48 h or other related malaria symptoms or history of malaria in the preceding two months. Children in the uncomplicated malaria category were those having parasitemia with fever (axillary temperature > 37.4 °C) and other classical symptoms such as headache, chill and sweating plus mild-moderate anemia (8 g/dL < hemoglobin < 11 g/dL), while severe malaria category were those with severe anemia (hemoglobin < 8 g/dL), fever plus one or more of other complications such as prostration, coma, jaundice, and respiratory distress [12].

2.2. Study procedure

2.2.1. Anthropometric measurement

Here, mid upper arm circumference (MUAC) was used as an anthropometric variable to assess the nutritional status of the enrolled children. At enrollment, MUAC of every child was measured to the nearest 1 mm at the midpoint between the elbow and the tip of the left shoulder by a trained research staff using the World Health Organization standardized Charcot’s strip. Children with MUAC < 125 mm were classified as having malnutrition while those with MUAC between 125 and 134 mm were classified as being at risk of malnutrition [13]. Children with MUAC measurements < 125 cm were further divided into two groups: those with severe acute malnutrition (MUAC < 115 mm) and those with moderate acute malnutrition (115 mm < MUAC < 125 mm) [13].

2.2.2. Parasite detection

Parasite was detected and identified by microscopic examination of thick (12 μL) and thin (3 μL) blood smear on grease-free labeled glass slides according to World Health Organization guidelines. The glass slides were prepared in duplicates and read by two independent trained microscopists. A glass slide was considered parasite negative after examining 200 high power fields without seeing one parasite. Parasitemia was measured by counting the number of parasites against 200–500 leukocytes using the thick blood film and expressed as number of parasites per one microliter of whole blood, assuming 8000 leukocytes per one microliter of blood. The thin blood film slides were used for speciation and all malaria positive slides showed the presence of the asexual stage of P. falciparum only.

2.2.3. Blood hemoglobin measurement

A drop of finger pricked blood drawn into a microcuvette was used for the determination of hemoglobin (g/dL) using a Hemocue machine (Hemocue Hb 201). Anemia was defined as hemoglobin below 11 g/dL and in this study, hemoglobin < 8 g/dL was taken as severe malaria.

2.2.4. Genomic DNA extraction

The extraction and purification of genomic DNA from peripheral blood samples of the study participants was performed using the salting out method described previously [11].
2.2.5. TLR4 (Asp299Gly and Thr399Ile) genotyping

The alleles and genotypes of the two studied TLR4 SNPs were determined by PCR-restriction fragment length polymorphism based method using the previously used primers sequences by Nyati et al. [14] (Table 1). All PCR amplification reactions were performed in a 20-μL volume PCR tubes containing 10x PCR buffer, 200 μmol/L each of the deoxynucleotide triphosphates, 2.0 mmol/L of MgCl₂, 20 pmol of each primer, 1.25 IU of Taq DNA polymerase (Promega, Madison, USA) and 100 ng of each genomic DNA as template. In a thermal cycler (Techne™ Thermal Cycler TC-312, Fisher Scientific, UK), the PCR reaction was then subjected to denaturation at 94 °C for 5 min, followed by 35 cycles of denaturing at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min and cooling to 4 °C. Template-free water was used as a negative control. After amplification, the purified 4-μL PCR products were subjected to restriction digestion by Nco I restriction endonuclease (Fermentas) for TLR4 Asp299Gly and by Hinf I for TLR4 Thr399Ile with 1 mL 10× enzyme buffer (Fermentas) overnight at 37 °C. The PCR products yielded 249 bp and 406 bp, respectively, while the digested products yielded same DNA fragments for the wild alleles but decreased to 223 bp and 377 bp fragments for the 299Gly and 399Ile mutant alleles, respectively, on 3% agarose gel after electrophoresis (Table 1). Each restriction enzyme assay was duplicated for the confirmation of restricted fragment length polymorphisms results.

2.3. Ethical consideration

The study protocol was performed according to the declaration of Helsinki and approved by the ethics committee of Lagos State Health Management Board. Informed consent was obtained from the caregiver or guardian of every child enrolled into the study.

2.4. Data analysis

Data from Ibeshe were excluded from this study due to lack of MUAC data. Only age and sex-matched samples with MUAC data were analyzed. Data are summarized as number and percentages for categorical variables, while mean with SD was used to summarize continuous variables. TLR4 genotype frequencies were calculated by counting. For categorical variables and TLR4 genotypes, frequency comparisons were done between infected and non-infected children, among malaria sub-groups and between the present study and previous studies from other malaria endemic countries using Chi-square test or Fisher’s exact test. For mean comparison, Student’s t-test (unpaired mean) and Duncan test were used for analysis. For every statistical test, an outcome with a P-value < 0.05 was considered to be significant.

3. Results

Baseline demographic and clinical characteristics of the studied children population are presented in Table 2. On the whole, the proportions of children with severe acute malnutrition, acute malnutrition and risk of malnutrition were 3.2%, 12.0% and 49.5%, while for severe anemia, moderate anemia and mild anemia were 3.2%, 6.0% and 24.5%, respectively. The P. falciparum infected children significantly elicited lower MUAC, higher rate of acute malnutrition and lower mean hemoglobin level compared to their non-infected controls. Among the infected children, parasitemia range of 900–102700 parasites per μL was found with parasitemia > 10000 parasites per μL seen in 12.7% of the infected children. Further stratification of the P. falciparum infected children identified 41 (51.9%), 33 (41.7%) and 5 (6.3%) as having asymptomatic malaria, uncomplicated malaria and severe malaria by case definition. The mean parasite densities of these malaria subgroups were 3150, 10970 and 71460 parasites per μL. In the infected children, significant (P < 0.05) decrease in blood hemoglobin level was observed as parasitemia increased from < 5000 parasites/μL through 5000–9999 parasites/μL to > 10000 parasites/μL. Contrastingly, the observed decrease in mean MUAC from 130.8 mm to 125.7 mm was not significant (P > 0.05) (Table 3). TLR4 genotyping of the studied population revealed 4 distinct patterns of TLR4 genotypes and Asp299Asp/Thr399Thr was found as the commonest co-segregated genotype with an overall prevalence of 90.2%. This was followed by Thr399Thr was found as the commonest co-segregated genotype and Asp299Asp/Thr399Thr genotype was observed in the severe malaria subgroup compared to other subgroups. For TLR4 Asp299Gly/Thr399Ile genotype, significantly higher prevalence

---

Table 1
Nucleotide sequences of primers used and amplification conditions.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence</th>
<th>Amplification profile temperature (°C)</th>
<th>Product size (bp)</th>
<th>Restriction enzyme</th>
<th>Cut product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>D  A  E  C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR4</td>
<td>299TLR4F</td>
<td>5'-GATTACGATACCTTAGACTACCTCATGATG-3'</td>
<td>94 55 72 35</td>
<td>249 Nco I (Fermentas)</td>
<td>W = 249 M = 223 + 26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>299TLR4R</td>
<td>5'-GATCAACTCTGAAAAAGCATGCCAC-3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>399TLR4F</td>
<td>5'-GTTGCTGTTTCAATAGTGATTTGGGAGAA-3'</td>
<td>94 55 72 35</td>
<td>406 Hinf I (Fermentas)</td>
<td>W = 406 M = 377 + 29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>399TLR4R</td>
<td>5'-ACCTGAGAGCGAGAGTGAATTAAGGCT-3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D: Denaturation; A: Annealing; E: Extension; C: No. of cycles; W: Wild allele; M: Mutant allele. The time for denaturation, annealing and extension was 30 s, respectively.
were analyzed by AM: Asymptomatic malaria; UM: Uncomplicated malaria; SM: Severe malaria. Differences in frequencies of genotypes among malaria subgroups

Table 5
Frequencies of TLR4 genotypes from Asp299Gly and Thr399Ile SNPs among P. falciparum infected children stratified into malaria subgroups [n (%)].

<table>
<thead>
<tr>
<th>TLR4 genotype</th>
<th>All (N = 79)</th>
<th>AM (N = 41)</th>
<th>UM (N = 33)</th>
<th>SM (N = 5)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp299Gly/Thr399Thr</td>
<td>7 (9.2)</td>
<td>0 (0.0)</td>
<td>7 (21.9)</td>
<td>0 (0.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Asp299Gly/Thr399Thr</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gly299Gly/Thr399Thr</td>
<td>2 (2.6)</td>
<td>1 (2.4)</td>
<td>1 (3.0)</td>
<td>0 (0.0)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

AM: Asymptomatic malaria; UM: Uncomplicated malaria; SM: Severe malaria. Differences in frequencies of genotypes among malaria subgroups were analyzed by Chi-square or Fisher’s exact test. P value less than 0.05 was considered to be significant.

4. Discussion
The availability of SNPs data of the Yoruba ethnic group from Nigeria (http://www.hapmap.org) [15] has provided a great opportunity to investigate candidate gene markers such as TLR4 in the pathogenesis of clinical malaria in the country. The 87.3% contribution to P. falciparum malaria infection by children aged < 5 years and 49.5% risk of malnutrition observed in the study population have further highlighted the enormous burden of malaria and malnutrition among children under 5 specifically and children of all age groups generally in Nigeria. Previous phenotypic studies have reported low socio-economic factors, malnutrition and poor access or use of malaria interventions as potential risk factors for clinical malaria in Nigerian children [16,17]. In this study, MUAC was measured to diagnose malnutrition in the studied children not only because it is easier and simple to perform but also because it shares good reliability with weight for height, weight for age and height for age z scores in diagnosing severe and moderate acute malnutrition in children [18]. In this study, MUAC value of the infected children did not decline significantly with significant rates of 97.6% and 87.9% were observed in asymptomatic malaria and uncomplicated malaria subgroups compared to 40% carriage in severe malaria children (P < 0.05) (Table 5).
increase in parasitemia. This may be due to a relatively lower 12.7% of infected children who elicited parasitemia > 10000 parasitemia compared to 57.0% of the infected children with parasitemia less than 5000 parasites/µL. The deleterious effect of significantly higher average parasitemia of 71 460 parasites/µL in severe malaria subgroup could also have been offset by the small number of cases (i.e. 6.3% of total infection) compared to 51.9% of asymptomatic malaria cases with a much less deleterious mean parasitemia of 3 150 parasites/µL in the dataset analyzed in this study. In our previous study in Takwabay, a pyrogenic threshold of > 3 500 parasites per µL during the rainy season was found among the P. falciparum infected children [19]. Our finding has also supported previous studies in which malnutrition was found not to impact on malaria morbidity [20]. However, further studies with a larger dataset for severe malaria and measurement of micronutrients such as zinc and vitamin A or macronutrients such as iron with inflammatory markers would be needed to clarify the present finding. The observed significant decline in hemoglobin level with increase in parasite density in this study is expected because of the parasite obligate requirement of iron and amino acids from hemoglobin for intracellular asexual growth and development in the erythrocyte, followed by hemolysis at the completion of schizogony. With higher level of parasitemia, more erythrocytes are likely to be invaded and lysed leading to hemoglobin loss, and anemia ensues [21]. Loss of erythrocytes and hemoglobin may also occur as a result of destruction of infected and non-infected erythrocytes by the reticuloendothelial systems in the spleen and liver, by sequestration of the infected erythrocyte in the vasculature and through rosetting [22].

This study found four out of the six possible TLR4 co-segregated genotypes that could arise from the combination of Asp299Gly and Thr399Ile SNPs. The identified SNPs were Asp299Asp/Thr399Thr, Asp299Gly/Thr399Thr, Asp299Gly/Thr399Ile and Gly299Gly/Thr399Thr. With the exception of the Asp399Asp/Thr399Thr, the three other SNPs lack occurrence in many East Asian countries, including Japan, Korea and China where much less cases of falciparum malaria occur [23–25]. The presence of these TLR4 genotypes also suggests the existence of local pressure offered by infectious diseases, including malaria, in Nigeria. Malaria plays an important role in the selection of these polymorphisms irrespectively of the intensity of transmission which can be exemplified by the occurrence of these TLR4 genotypes together with the Asp299Asp/Thr399Ile among the Baluchi and other ethnic groups in Iran where only cases of mild malaria are seen [26–27]. In terms of individual TLR4 genotypes, the Asp299Gly/Thr399Ile genotype is specifically absent in Asia but highly present in Europe, while the Asp299Gly/Thr399Thr is highly present in Africa [25,26]. The extreme occurrence of these genotypes may be attributed to high number of cases of sepsis/septic shock in Europe and severe malaria cases in Africa [25,28]. The Asp299Gly SNP is associated not only with the poor glycosylphosphatidylinositol inflammatory response in the early phase of malaria but also with poor handling of lipopolysaccharide endotoxin from Gram negative bacterial pathogens thereby promoting sepsis and septic shock. However, in Europe, this SNP is in high linkage disequilibrium with Thr399Ile, which protects against sepsis and septic shock [25,28]. This explains why the frequency of Asp299Gly/Thr399Ile co-segregated genotype is high in Europe. Unlike in Europe, the level of co-segregation of the Asp299Gly and Thr399Ile SNPs is low in Africa [25,28]. Given the fact that the Asp299Gly also supports severe cases of malaria, which occurs highly in Africa, the high frequency of the Asp299Gly/Thr399Thr has become inevitable.

In terms of frequency, the disparity in occurrence for each of the TLR4 genotypes identified in this study was found to be non-significant between infected and non-infected children. This result suggests that by pooling malaria cases based on infection instead of disease or clinical manifestation of malaria, the role played by TLR4 genotypes among the Nigerian Yoruba children population may not be apparent. This result may also be a reflection of the high level of malaria exposure in Nigeria where every child remains at risk of malaria infection. The microscopic method used for the diagnosis of malaria in this study lacks the sensitivity of PCR to detect lower parasitemia, which cannot be ruled out in the control children. However, our result is in consonance with that of Pirahmadi et al. [26]. The workers also found no significant disparity in the distribution of these genotypes between infected and non-infected Baluchi children in Iran where the level of malaria exposure has been low. Although the present study was conducted during the early rainy season into the high malaria transmission season, a declining malaria exposure cannot also be ruled out due to the recent high intensity malaria control activity offered by the Eko Malaria Project that is characterized by significant improvement in long-lasting insecticidal nets and artemisinin-based combination therapy coverage in the study area [29]. The limited number of age and sex-matched severe malaria cases (n = 5) in the pool of infected and non-infected children (n = 184) analyzed in this study may also offset the expected association of some of the identified TLR4 genotypes with malaria infection. In support of this possibility are the results obtained when TLR4 genotype data from only the infected children were analyzed. This study found Asp299Asp/Thr399Thr to be the most occurring TLR4 genotype among the infected children as a whole with a prevalence of 89.9%. This further increased to 97.6% among asymptomatic malarial children and high still at 87.2% in uncomplicated malaria subgroup compared to 40.0% in severe malaria subgroup. This result supports the protective role of this genotype against severe malaria as reported by previous studies [7,9,11,25]. High carriage of Asp299Asp/Thr399Thr has also been reported among Baluchi children at 80.6%, Sudan at 90.1%, Cameroon at 93.3%, the Dogon ethnic group in Mali at 91.1%, the Mali Fulanis at 97.5% and the Han Chinese at 100% [25,26]. With only Sudan having similar level of occurrence, the frequency variation observed for Asp299Asp/Thr399Thr genotype between Nigeria and other malaria endemic countries analyzed was found to be significant. Although Asp299Asp/Thr399Thr is protective against severe malaria and occur highly in malaria endemic African and Asian countries, its local selection by infectious diseases can be said to be country or geographical location dependent. Apart from malaria, other infectious diseases that select for this polymorphism may vary in occurrence between Nigeria and other countries. Such infectious diseases include respiratory syncytial virus, Candida albicans, Salmonella typhi, Mycobacterium tuberculosis and Neisseria meningitidis [30–33]. The second commonest TLR4 genotype found among the infected children in this study was the Asp299Gly/Thr399Thr genotype at 7.5% among the infected children as a whole. This genotype was found at 9.1% among uncomplicated subgroup and significantly at 60% among severe malaria subgroup. In agreement with previous studies [9,11,25]
and because of its absence among the asymptomatic subgroup, the present study has further implicated the Asp299Gly/Thr399Thr genotype in susceptibility to severe malaria. Larger sample size would be needed to validate this susceptibility role among Nigerian children. However, in our recent study, we found the Asp299Gly SNP occurring in 17.6% of children with severe malaria [11]. But this SNP was not investigated in a co-segregated state as shown in the present study. Also in a co-segregated state and among Ghanaian children, Mockenhaupt et al. [9] also reported a significant association of this SNP with severe malaria. A further clue to the possible role of the Asp299Gly/Thr399Thr genotype is its higher occurrence at 8.3% among the Dogon tribe of Mali who are naturally more susceptible to severe malaria, but at a lower 2.5% occurrence among the Fulani ethnic group of Mali who are naturally less susceptible to severe malaria [25]. In some other malaria endemic countries, the reported rates of the genotypes are 3.4% in Iran, 5.8% in Tanzania, 6.3% in Cameroon and 9.4% in Sudan. This co-segregated genotype is absent in the Han Chinese population [25]. The Asp299Gly/Thr399Ile genotype is well known for its protective role against severe malaria in Africa and sepsis in Europe [25,28]. Therefore, it is not surprising to have a low rate of occurrence at 1.3% among the infected children and 1.6% in the whole study population. Lower rate of occurrence of this genotype has previously been reported in Sudan at 0.5%, in Mali at 0.4% among the Dogon ethnic group and in Cameroon at 0.4% [25]. However, higher rates at 1.7% and 6.6% have also been reported in Tanzania and Iran [25,26]. Lastly, this study found the Gly299Gly/Thr399Thr genotype to occur only in uncomplicated malaria children at 1.3% and at higher rate of 5.7% among non-infected children. The 1.3% occurrence rate of the Gly299Gly/Thr399Thr genotype is comparable to 1.4% reported among Baluchi children with mild malaria. However, unlike in Baluchi non-infected children, an occurrence rate of 5.7% for this genotype was found in this study among the non-infected children. This co-segregated genotype has been associated with an increase in susceptibility to malaria but a reduction in mortality and cerebral malaria in Ghanaians and Cameroonians 9,25].

This study has a limitation of small sample size, most especially for severe malaria subgroup compared to other malaria subgroups. This could be attributed to the design of this study as only data from sex and age-matched case and control subjects were analyzed. Another limitation is the non-use of molecular method to diagnose malaria as this would have not only improved the detection of the malaria parasites but also validate the parasitic negative status of children in the control group. Therefore, future studies with a larger sample size and use of PCR-based method for malaria diagnosis are needed to further clarify the present findings.

Based on the findings from this study, we report diversity of malaria associated TLR4 genotypes in Nigerian children with Asp299Gly/Thr399Ile and Asp266Asp/Thr399Thr co-segregated genotypes playing a protective role against severe falciparum malaria.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors wish to thank the health workers at the study centres for their technical support. The financial support of Iru Local Government Chairman is also acknowledged. Primers and restriction enzymes used for this work were procured through self-funding by members of the research team.

References


