Seizures in children with acute falciparum malaria: risk factors, mechanisms of neuronal damage and neuro-protection
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Chapter 3

Haptoglobin HP2-2 Genotype, α-Thalasaemia and acute seizures in children living in a malaria endemic area of Kenya

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Submitted
ABSTRACT

Background
Polymorphisms of the haptoglobin (HP) gene and deletions in the α-globin gene (α-thalassaemia) are both found at high frequencies in many parts of malaria-endemic Africa. The same regions also have very high incidence rates for childhood acute seizures. We investigated the hypothesis that the HP2-2 genotype and the common African α-globin gene deletions are associated with an increased risk of seizures in children.

Methods
Two hundred and eighty eight children aged 3–156 months, consecutively admitted to Kilifi District Hospital in coastal Kenya with acute seizures were matched for ethnicity to an equal number of community controls. We compared the proportions of cases and controls with the HP2-2 genotype and the common African 3.7kb α-globin gene deletions.

Results
The proportion of cases (72/288 [25.0%]) and controls (80/288 [27.8%]) with the HP2-2 genotype was similar, p=0.499. The allele frequency of the HP2 gene in cases (49.3%) and controls (48.6%) was also similar, p=0.814. Similarly, we found no significant difference between the proportion of cases (177/267 [66.3%]) and controls (186/267 [69.7%]) with deletions in the α-globin gene (p=0.403). Among cases, the HP2-2 polymorphism and deletions in the α-globin gene were neither associated with a change in the type (focal or generalized), number or duration of the seizures nor did they affect the outcome of treatment.

Conclusions
Polymorphisms in the haptoglobin gene and deletions in the α-globin gene are not a risk factor for acute seizures in children. Future studies should examine other susceptibility genes.
INTRODUCTION

Seizures are the commonest neurological symptom in sick children admitted to hospital. In parts of malaria-endemic Africa, over 15% of paediatric admissions have a history of seizures[1-3] and acute seizures are a major risk factor for epilepsy later in life[4, 5]. Most seizures occur in febrile children and are precipitated by infections[2, 3, 6] but in some children, genetic factors may be important[2, 7].

Polymorphisms of the haptoglobin (HP) gene and deletions in the α-globin gene (α-thalassaemias) are very common in malaria endemic Africa[8, 9] and both are associated with protection against malaria [8-11]. The same region also has high incidence rates for childhood acute seizure disorders [1, 12]. It is unclear whether these polymorphisms or deletions and the high incidence of seizures are associated.

Haptoglobin is a protein that binds free haemoglobin in circulation. The gene for this protein has 3 main polymorphisms: HP1-1, HP1-2 and HP2-2. Studies have associated the HP2-2 genotype with idiopathic generalized epilepsy[13, 14] and the mean plasma levels of haptoglobin in patients with idiopathic epilepsy is lower than that in normal population controls[15]. It has been suggested that in persons with the HP2-2 genotype, a defective free haemoglobin clearing system after central nervous system micro-hemorrhage events is involved in the development of chronic seizure disorders[14, 15].

The relationship between the α-thalassaemias and acute seizures has not been reported. It is postulated that, children with thalassaemia have an increased oxidative stress on neurons and an altered iron metabolism. The change in iron metabolism may interfere with activities of gamma amino butyric acid and the glutamate system[16] and alter the risk for seizures. Only one retrospective study has examined the incidence of seizures in children with β-thalassaemia and the incidence was lower than that in the general population[16].

We hypothesized that compared to children with HP1-1 and HP1-2 genotypes or those without deletions in the α-globin gene, children with HP2-2 genotype or the common African α-globin gene deletion have an increased risk of developing acute seizures. We recruited children hospitalized with acute seizures and compared their HP genotypes and α-thalassaemia types to that of a matched cohort of community controls.
MATERIALS AND METHODS

Study participants
The study was conducted in Kilifi District on the coast of Kenya where most residents are collectively known as the Mijikenda, a Bantu grouping of nine tribes, with the Giriama (45%), Chonyi (33%), and Kauma (11%) forming the major groups. A system of continuous demographic surveillance (DSS) is maintained in a population of roughly 230,000 living in the immediate catchment area of Kilifi District hospital[17]. Cases were children from within the DSS aged 3–156 months, consecutively admitted to the paediatric wards of the hospital with acute seizures. Children with epilepsy (2 lifetime episodes of unprovoked seizures) were excluded. Controls were children born in the hospital who had cord blood samples collected and archived, and were frequency matched to cases on the basis of ethnicity. The main exposure factor of interest was haptoglobin HP2-2 genotype. We estimated that a sample size of 248 cases and 248 controls would give 90% power to detect an OR of 2.0 at 5% level of significance given a prevalence of HP2-2 in the community of 25%[8]. Ethical approval for the study was granted by the Kenya Medical Research Institute.

Procedures
The cases received emergency care and resuscitation based on standard guidelines[18]. Parents were then invited to participate in the study and consent obtained. The history included the number, duration, and a description of the seizures and a history of previous seizures in the child. Level of consciousness was assessed using the Blantyre Coma Scale[19]. Specific anti-microbial therapy was given for malaria and for bacterial infections. At discharge, all patients were assessed for the presence of neurological deficits.

Venous blood was drawn and plasma from both cases and controls was immediately separated by centrifugation and the cell pellet frozen and stored at -80°C. DNA was extracted using commercially available kits according to the manufacturers instructions (PUREGENE® DNA extraction kit, GENTRA SYSTEMS, Boston, MA) and haptoglobin and thalassaemia genotyping was performed by PCR as previously described[9, 10, 20].

STATISTICAL ANALYSIS
We compared the prevalence of HP2-2 genotypes, α-thalassaemia deletion types and allele frequency in cases and controls using Pearson’s chi square test to investigate the association with acute seizures in children. In addition, we compared the seizure types
(generalized, focal or secondarily generalized) and the median number of seizures in children with the different genotypes. We also performed a sub analysis comparing the genotypes of cases with *Plasmodium falciparum* infection.

**RESULTS**

Three hundred and twenty five children, consecutively admitted with acute seizures to Kilifi District hospital, between December 2004 and August 2005 were recruited. Haptoglobin genotyping was successful in 294 (90.5%) of whom, 288 were frequency matched for ethnicity to an equal number of controls from the birth cohort (six cases did not have matching ethnic groups in the birth cohort). In 267 out 288 (92.7%) children, both the haptoglobin genotype and α-thalassaemia type was available and the cases and controls could be matched for ethnicity. The majority of cases, 167 (58.0%) were from the Giriama ethnic group, 67 (23.3%) were Chonyi, 32 (11.1%) Kauma while the remaining 22 cases belonged to the Digo, Duruma, Jibana, Kambe, Rabai or the migrant Luo ethnic groups.

The median (IQR) age of the cases was 25.6 (14.0 - 41.1) months and 147 (51.0%) were males. The majority, 276 (95.8%), presented with fever and 104 (36.1%) reported previous episodes of provoked seizures. Malaria was the leading primary diagnosis associated with acute seizures and was seen 186 (64.6%) cases. Other primary diagnoses included respiratory tract infections, otitis media, acute diarrhoea or childhood viral fevers in 78 (27.1%), acute bacterial meningitis/septicaemia in 7 (2.4%) and suspected viral meningoencephalitis in 7 (2.4%). Ten children (3.5%) had other encephalopathies.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Haptoglobin genotypes in children with and without acute seizures</th>
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<tr>
<td><strong>Subject group</strong></td>
<td><strong>Haptoglobin genotypes</strong></td>
</tr>
<tr>
<td></td>
<td><strong>HP1-1, n (%)</strong></td>
</tr>
<tr>
<td>Cases</td>
<td>76 (26.4)</td>
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<tr>
<td>Controls</td>
<td>88 (30.5)</td>
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<th>Table II</th>
<th>α-thalassaemia genotypes in children with and without acute seizures</th>
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<tr>
<td><strong>Subject group</strong></td>
<td><strong>α3.7-kb globin deletion types</strong></td>
</tr>
<tr>
<td></td>
<td><strong>No deletion, N (%)</strong></td>
</tr>
<tr>
<td>Cases</td>
<td>90 (33.7)</td>
</tr>
<tr>
<td>Controls</td>
<td>81 (30.3)</td>
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</table>
No significant association was found between seizures and haptoglobin HP2-2 genotype (table I). The allele frequency of the HP2-2 genotype in cases and controls was similar ($\chi^2=0.57, p=0.499$). Similarly, we found no association between acute seizures and $\alpha$-thalassaemia (table II). The proportion of cases and controls with any deletion in the globin gene was similar, ($\chi^2=0.70, p=0.403$).

A parental description of the seizure event was available in 278/288 cases; the seizures did not occur in the presence of the consenting parent in 10 cases. Two hundred and thirty four children (84.2%) had generalized seizures, 37 (13.3%) had focal seizures while 7 (2.5%) had secondarily generalized seizures. HP2-2 genotype and deletions in the $\alpha$-globin gene were not associated with any particular seizure manifestation, number of seizures, presentation with status epilepticus or with impairment of consciousness. In addition, there was no association between the HP2-2 polymorphism or deletions in the $\alpha$-globin gene with malaria related seizures or outcome (table III).

### DISCUSSION

Increasingly, genetic factors are being recognised as important risk factors for both acute (febrile seizures, reviewed in [21]) and chronic (epilepsies, reviewed in [22]) seizure disorders. Studies have demonstrated a strong association between the haptoglobin HP2-2 polymorphism and idiopathic generalized epilepsies[13, 14]. In this study we
investigated whether this risk extends to acute seizures and whether another common genetic trait in Africa - a 3.7kd deletion in the α-globin gene - is associated with the high incidence of acute seizures in this region. The findings do not support our hypothesis: the prevalence of the HP2-2 genotype and α-thalassaemia genotypes were similar in cases and controls; the median number of seizures during the current illness and the frequency of past seizures in children with these polymorphisms/deletions were similar to that in cases without the polymorphisms/deletions.

Accumulation of iron and iron containing products such as haemoglobin in the interstitial tissues of the brain can initiate and enhance the generation of reactive oxygen species and cause neuronal damage by peroxidation of cell membrane lipids[15, 23]. Haptoglobin is an acute phase protein, which binds free haemoglobin released into circulation following haemolysis. Experimental evidence suggests that hypo-haptoglobinemia is associated with poor clearance of free haemoglobin from the central nervous system and may lead to seizure disorders [15]. It was postulated that the inefficient clearance of free haemoglobin in patients with HP2-2 genotype increases unbound free haemoglobin in the brain (and other tissues) and may be associated with increased haemoglobin mediated oxidant stress on cell membranes, oxidative neuronal damage and an increased risk of seizures. In addition, α-thalassaemia deletions may affect a child’s susceptibility to acute seizures due to possible changes in neurotransmitter metabolism. Our findings suggest that, unlike the idiopathic generalized epilepsies in which the HP2-2 genotype may be one of many contributory genes involved in the polygenic inheritance of the seizure disorder[13, 14], this polymorphism is not a risk factor for acute seizure disorders.
Although we did not measure plasma haptoglobin levels to directly correlate genotype with phenotype, the evidence for the lack of association between HP2-2 genotype and acute seizures is compelling. It is possible that there is a time lag between the onset of free haemoglobin induced “oxidative neuronal damage” and manifestation of the seizure disorder i.e. epilepsy. Alternatively, repeated episodes of “neuronal oxidative damage” are necessary before the seizure disorder can develop.

Despite this finding, family studies in children from this community do provide strong evidence to support a genetic risk factor for acute seizures [7]. It is likely that there are other susceptibility genes. Mutations and single nucleotide polymorphisms in voltage and ligand gated channels such as those described in children with febrile seizures or generalized epilepsy with febrile seizures plus may be possible candidates [24-26]. Screening for these genetic disorders may be a useful step.

CONCLUSION

In conclusion, the haptoglobin HP2-2 genotype and deletions in the α-globin gene are not risk factors for acute seizures in Kenyan children with and without falciparum malaria infection. Future studies should examine other susceptibility genes.

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