Introduction
The number of episodes of clinical illness caused by the malaria parasite *Plasmodium falciparum* worldwide had been underestimated for years but was recently corrected to an estimate of 515 million episodes of clinical malaria per year (1).

In contrast to the number of studies on clinical malaria complications, parasitaemia or mild clinical malaria have not been analysed in great detail regarding the influence of human genetic factors. The major reason may be that they require laborious and expensive longitudinal assessment of phenotypes. In addition, mild clinical malaria is difficult to investigate in endemic areas where virtually all children frequently carry malaria parasites in their blood and show only statistically elevated parasitaemias during malaria attacks (2). The parasitaemia of *Plasmodium* trophozoites is central to malaria pathogenesis, transmission and acquired immunity. The classical malaria resistance factors of haemoglobin (Hb) S and C have been shown to have protective effects on parasitaemia and mild clinical malaria (3,4).

Segregation analyses of parasitaemia and mild disease episodes showed a complex inheritance model, whereby the predominant effect of genetic factors was on childhood malaria (5,6). Two previous linkage studies have addressed candidate genomic regions. They provided and supported, respectively, evidence for linkage to the cytokine-gene cluster on human chromosome 5q31-q33 (7,8).

We conducted a family-based longitudinal study to perform genome-wide linkage analyses on the phenotypes of mild clinical malaria and blood-stage parasite density. Additional phenotypes including anaemia and spleen enlargement as well as the sampling of parasite isolates were included to further refine the malaria phenotypes.

Project Status

Linkage study
A study cohort has been selected, phenotyped and subjected to genome-wide marker analysis in DHGP/NGFN1.

(i) Selection: 2652 parental pairs with at least 3 offspring aged 0.5-12 years resident in a hyperendemic malaria area in Ghana, West Africa, were screened not to segregate any of the known or presumed malaria resistance factors of Hb S and C, alpha-thalassaemia 3.7" and glucose-6-phosphate-dehydrogenase deficiency A. 129 families fulfilled the criteria (463 offspring in total). During phenotyping for malaria, malaria protection by window screens or the use of bednets was recorded. Since the group protected by window screens or the use of bednets was not significantly lower parasitaemias, it was excluded from further analyses. Prior to malaria phenotyping, all children were treated for hookworm infection and with iron supplementation.

(ii) Phenotyping: The children were followed over 31 weeks during an entire rainy season including weekly reports of clinical episodes suggestive of malaria and malaria treatments, temperature recordings and parasite counting as well as biweekly haematocrit determinations (Fig. 1). The biweekly blood specimens were preserved. With 14,294 out of 14,415 total attendances, compliance was > 99%. So far, parasitological phenotypes and phenotypes for clinical illness clinical have been defined.

(iii) Genetic study group: Familial relationships have been tested using short-tandem-repeat (STR) markers. Exclusion of families or children with incompatibilities resulted in 105 nuclear families with 372 siblings (502 sibpairs) of whom both parents are available for genotyping (582 individuals in total). These can be investigated for linkage, association and haplotype sharing with quantitative traits.

(iv) Genotyping: A subgroup of 376 individuals comprising 240 siblings (328 sibpairs) from 68 families have been selected according to their phenotypic variability and included for genome-wide typing using 10,000 genome-wide SNP markers (Affymetrix). The scan was performed at the DHGP/NGFN genotyping platform.

(v) Preliminary results: A number of five genomic regions with significant linkage (Z-scores of modell-free linkage > 3.5) for phenotypes of parasite densities and episodes of clinical illness were identified. In addition the most promising linkage region for the latter phenotype was confirmed (Z-score > 3.0) in the complete study group by the use of 20 STRs. In preparation for a gene identification procedure a number of 5000 verified SNPs (with emphasis on coding SNPs, SNPs with from splice sides and those from promoter or 3’ untranslated regions) were selected from public databases (NCBI, Perlegen Sciences, and CHIP Bioinformatics Tools) in a 2.15 Z support region (equivalent to...
a LOD of 1.0). At present a gene identification procedure in the genomic region that will yield most significant evidence for linkage with clinical malaria illness is conducted. It will comprise the genotyping of 1000 SNP loci at the NGFN genotyping platform.

A new test for alpha\(^+\)-thalassemias

Thalassemias are among the most common monogenic disorders of humans. They are characterized by a reduced synthesis of one of the hemoglobin protein chains and cause anemia. Alpha-thalassemias result in the vast majority of cases from large deletions at the alpha-globin locus. The alpha\(^+\)-thalassemias are characterized by deletions that inactivate both alpha-globin genes (HBA) of a given chromosome, whereas in alpha-thalassemias, one gene remains functional.

Existing genotyping methods rely on Southern blot hybridization, which is laborious and expensive, or use gap PCRs, which have some limitations due to (i) the amplification of long GC-rich sequences, (ii) the requirement of the use of deletion-type specific primers and (iii) the risk that alleles may be amplified from minimal contaminations. Both methods could not easily be adapted to high throughput applications. Quantitative PCR has been proposed to avoid these problems. But, because of limited sensitivity, however, real-time PCR approaches have been successful only for the diagnosis of alpha-thalassemias.

Combining this assay with the previously described real-time PCRs for the diagnosis of alpha\(^-\) thalassemias may become the method of choice for the diagnosis of alpha-thalassemias.

**Outlook**

It is anticipated that the positional cloning procedure will result in the identification of additional genetic variants in candidate genes by re-sequencing. Genetic variants which show association with the phenotype in QTDT tests in this study will be confirmed by analysing a pilot study and will in addition be tested as to whether there is an effect on severe and complicated malaria in an other NGFN project. The identification of novel malaria resistance factors could unravel pathways involved in the pathophysiology of malaria, which would be of global medical importance.


Members of the study team include Edmund Browne, Yeetey Enuameh, Jennifer Evans, Birgit Foerster, Rolf Horstmann, Andre Kleensang, Inke Konig, Kingsley Osei Kwakye, Birgit Muntau, Peter Nurnberg, Ernest Opoku, Frank Ruschendorf, Jurgen Sievertsen, Andreas Ziegler, and many more. Photography: Mika Väisänen (www.mika-photography.de)