Polymorphisms in the genes of interleukin 12 and its receptors in association with protection against severe malarial anemia in children residing in western Kenya

[1,2]Lyna Zhang, [2]Donald Prather, [2]Jodi Vanden Eng, [2]Sara Crawford, [3]Simon Kariuki, [4]Feiko ter Kuile, [2]Bernard Nahlen, [2]Altaf A Lal, [2]Venkatachalam Udhayakumar, [2]Ya Ping Shi.
1) National Office of Public Health Genomics, Coordinating Center for Health Promotion; 2) Malaria Branch, Division of Parasitic Diseases, National Center for Zoonotic, Vector-Borne & Enteric Diseases, Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30341; 3) Kenya Medical Research Institute, Kisumu, Kenya; 4) Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK.

Background:

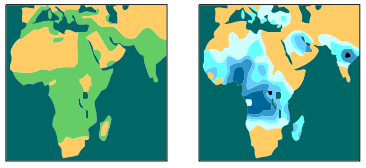
Of the more than 1 million Africans who die from *Plasmodium falciparum* infection each year, most die from severe malarial anemia (SMA) in children under five years of age. The considerable evolutionary pressure has put significant selection pressure on the human genome to select for mutations conferring protection against severe forms of malaria, such as sickle cell allele (Figure 1). Malarial anemia is characterized by the destruction of malaria-infected red blood cells and the suppression of erythropoiesis. *Interleukin 12* (*IL12*) significantly boosts erythropoietic responses in murine models. Its production was suppressed in children with SMA compared to asymptomatic children in Africa. For these reasons, the genes encoding the two *IL12* subunits, *IL12A* and *IL12B*, and its receptors, *IL12RB1* and *IL12RB2*, are attractive candidate genes for studying SMA.

Aims:

To determine whether gene polymorphisms of *IL12* and its receptors are associated with increased risk of developing the following related malarial morbidity outcomes in a cohort of children in western Kenya:

- Any severe anamia (Hb < 6 g/dL)
- High desity parasitemia (> 10, 000/uL)
- Severe anemia with any level of *P. falciparum* parasitemia (Hb < 6g/dL and parasite density > 0)
- Severe malarial anemia (Hb < 6 g/dL and parasitemia > 10,000/µL)

Figure 1: Malaria distribution correlates with the prevalence of the sickle cell mutation



Maps courtesy of evolution.berkeley.edu

Methods:

Study population - Asembo Bay Cohort, Kenya

- Holoendemic area of western Kenya
- 100-300 infective bites per person per year
- Longitudinal study, 1992-1999
- Blood samples taken from mother-infant pairs & other siblings <5 years old once per month until 5 years old
- Malaria parasitaemia treated with SP
- 940 unrelated individuals

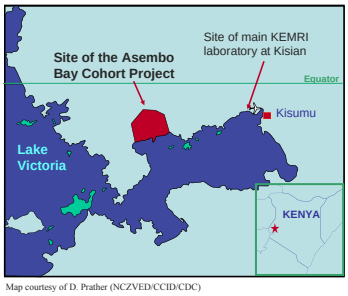
Genotypic Analysis

- A total of 75 tagging single nucleotide polymorphisms (tagSNPs) covering these four genes were included in this study
- Genotyping was performed with the Sequenom high throughput iPLEX MALDI-TOF mass spectrometry single base primer extension assay.

Statistical Analysis

- Using SAS 9.1 for Windows
- Where possible, longitudinal clinical information up to 2 years following study enrollment at birth has been used.
- Using univariate methods followed by multivariate analysis controlling for potential confounders of sickle cell, bednet use, treatment with anti-malarial drugs, amount of rain in the previous 120 days, placental malaria in the mother, and low birth weight (<2500g) or pre-term birth (<37 weeks gestation).

Figure 2: Location of Asembo Bay Cohort and CDC/ Kenya Medical Research Institute (KEMRI) Field Station in Western Kenya



Map courtesy of D. Prather (NCZVED/CCID/CDC)

Figure 3a: *IL12A* SNPs detected

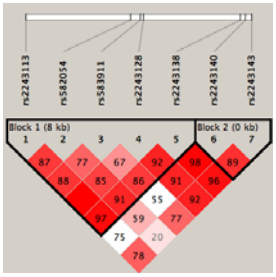
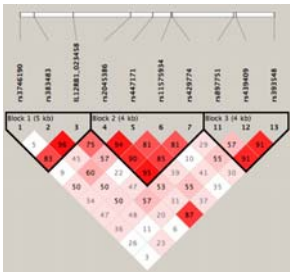


Figure 3b: *IL12RB1* SNPs detected



Results:

- Susceptibility to severe anemia with any level of parasitemia
  - Individuals possessing two copies of *IL12A* common allele (rs2243113) at promoter region, P = 0.009, RR 1.85, 95%CI 1.10-3.10
- Protection against high density parasitemia
  - Individuals possessing two copies of *IL12RB1* rare allele (rs383483), P = 0.003, RR 0.72, 95%CI 0.57 – 0.91
- Protection against severe malarial anemia
  - Individuals possessing two copies of *IL12RB1* rare allele (rs383483), P = 0.009, RR 0.49, 95%CI 0.26 – 0.92
  - Individuals possessing two copies of *IL12RB1* rare allele (rs429774), P = 0.0001, RR 0.18, 95%CI 0.05 – 0.72
- Susceptibility to severe malarial anemia
  - Individuals possessing two copies of *IL12A* rs2243140 common allele, P = 0.008, RR 3.42, 95%CI 1.21 – 9.62

Conclusions:

- Polymorphisms in the genes of *IL12* and its receptors are associated with protection against SMA in children in western Kenya
- Identification of genetic polymorphisms that influence human host susceptibility to malaria infection and severe disease outcomes may help us to better understand the immune response to malaria and design novel treatments against SMA.