Human genetic determinants of severe malaria in The Gambia

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About this study

Genetic factors play an important role in resistance to malaria infection but many of the genes responsible remain unknown. It has been estimated that host genetic factors contribute 25% of total malaria protection (Mackinnon MJ et al, 2005) but only 2% of this total can be attributed to HbS; a polymorphism which confers a well-documented protective effect against severe malaria. This raises the question: What other genetic variants have evolved that contribute to malaria protection or risk? In The Gambia, we have been building a DNA resource since 1996 with the aim of investigating the human genetic determinants of severe malaria.

Summary

An unmatched case-control study was conducted between 1996 and 2009 with the aim of investigating the human genetic determinants of severe malaria in The Gambia.Severe malaria cases (children aged between 4 months and 15-years) were recruited from the Western Health Region of the Gambia, the main referral hospital in Banjul, the MRC wards in Fajara, and some governmental health facilities. Clinical data and DNA were collected from both cases and controls. In about 50% of the cases, we collected DNA samples from both parents to allow a family-based (TDT) analysis. Controls were collected to represent the population of the Gambia; blood was collected from umbilical cords at the maternity units of the main hospitals. As with the cases, we also collected parental samples (mothers only) in about 50% of the samples.

Clinical data and DNA samples were contributed to the MalariaGEN Consortial Project 1 (CP1) along with those of 11 other case-control studies from a total of 11 malaria-endemic countries. As part of the sample handling process, baseline genotyping data was generated for a number of malaria–associated single nucleotide polymorphisms (SNPs) and the appropriate data has been returned to each site for site-specific analysis. A total of 69 SNPs at candidate genes (selection based on previous reports of association with severe malaria or on their likely biological role in malaria infection/disease) will be included in our analysis. We will investigate the association between each SNP and several malaria phenotypes including severe malaria, cerebral malaria, severe malarial anaemia, respiratory distress and hyperparasitaemia.

Study site description

The Medical Research Council (MRC) Unit in The Gambia recruited severe malaria cases, healthy controls and parents from the western part of the country, including the Kanifing Municipal Council (KMC) with a population of 397,327, and the West Coast Region, with a population of 527,753. This catchment had a total population of 956,396 in 2009 (2009 population projected from 2003 Population Census). Some cases and their parents were also referred from the North Bank West Region which has a total population of 92,822. The four largest ethnic groups in The Gambia are the Mandinka, Jola, Fula and Wollof. About half the inhabitants of the Western Health Region (WHR) are farmers, relying on their agricultural produce for subsistence. The GDP per capita for the country in 2009 was USD $436 (World Bank, accessed 12 Dec 2011).

The WHR is served by twelve government health facilities (in and out-patient) and four hospitals. In addition, there are 12 government-operated clinics (out-patient only) in the region. The MRC Clinical Services Department, Ahmadiyya Hospital and several private clinics in the study area also provide health care to the inhabitants in this region.

Transmission of malaria in The Gambia is seasonal. The long dry season lasts from November-June with a short rainy season from July-October with an average annual rainfall of 1,015 mm. Morbidity and mortality from malaria follows this pattern; both occur more frequently during the rainy season with a peak in October. *P. falciparum* is the dominant species, being responsible for all severe disease and for over 95% of clinical attacks. Few cases of clinical malaria are caused by *P. malariae*. The main vector species in the WHR are *Anopheles gambiae*, *A. melas* and *A. arabiensis*.

Methods

An unmatched case-control study, including parents of cases and mothers of controls was conducted between 1996 and 2009 recruiting patients admitted to the Edward Francis Small Teaching Hospital (EFSTH), formerly the Royal Victoria Teaching Hospital (RVTH), and the Medical Research Council (MRC) wards. The EFSTH is located in the capital, Banjul, and is The Gambia's main referral hospital. Patients come from all over the country, with the majority from the western region. The MRC wards are located in the main research site of the MRC in Fajara.

Cases consist of children (aged 4 months-15 years) with severe malaria. Severe malaria was defined by the presence of asexual parasitemia and any of the following: cerebral malaria (Blantyre coma score of ≤2), severe anaemia (haemoglobin <6 g/dl or hematocrit <18%), respiratory distress (respiratory rate >40 with 2 of the following: nasal flaring, intercostal and subcostal recession, and grunting), repeated generalised convulsions, hypoglycaemia (blood glucose <2.2mmol/l or <40mg/dl), renal failure (urine output <12ml/kg/day), haemoglobinuria, jaundice, prostration, hyperparasitaemia (≥500 parasites/HPF), hyperpyrexia, circulatory collapse, cold extremities, rapid pulses, systolic blood pressure below 50mmHg.

Controls consist of cord blood samples recruited from various labour wards, primarily in the western division of The Gambia, between 1999 and 2007. Blood samples were collected from the parents of a number of severe malaria cases, and mouth swab samples from mothers of a number of controls

A standardised case report form (CRF), was created for the Consortial Project 1 and used by all sites to collect standardised clinical data. The data collected in Gambia (and all other sites) was uploaded

onto secure web-based software developed by MalariaGEN. Here, the integrity of the data was checked and data was standardised and amalgamated.

Genomic DNA was extracted at the MRC Unit in The Gambia from whole blood using the Nucleon™ BACC2 Genomic DNA extraction kit® (Gen-Probe Life Sciences Ltd., Manchester, UK) using manufacturer's instructions. Aliquots of the DNA samples were shipped to the MalariaGEN Resource Centre in Oxford for further processing and quality control for quantity, quality (by genotyping) and confirming appropriate clinical data was available, prior to selection for the genome-wide experiment. Baseline genotype data for 69 malaria-associated SNPs was generated for all contributing samples; briefly, samples underwent a primer-extension pre-amplification (PEP) step (Xu K et al, 1993; Zhang L et al, 1992) prior to genotyping on the Sequenom® MassArray® platform. Following curation, the genotype data were returned to The Gambia to allow local analyses.

Table 1: Breakdown of samples

Number	Gender: n (%)	Age in years: n (%)	Ethnicity: n (%)
Malaria cases: 2,801	Male: 1429 (51) Female: 1296 (46) Not recorded: 87 (3)	<1: 236 (8) 1-2: 405 (14) 2-5: 1336 (48) 5-15: 813 (29) Not recorded: 11 (<1)	Fula: 344 (12) Jola: 432 (15) Mandinka: 900 (32) Wollof: 335 (12) Other: 780 (28) Not recorded: 10 (<1)
Healthy controls: 4472	Male: 1979 (44) Female: 2315 (51) Not recorded: 221 (5)	<1: 3801 (85) 1-2: 0 (0) 2-5: 2 (<1) 5-15: 17 (<1) >15: 211 (5) Not recorded: 441 (10)	Fula: 784 (18) Jola: 527 (12) Mandinka: 1325 (30) Wollof: 597 (13) Other: 519 (12) Not recorded: 359 (8)

Ethics

The study was approved by The Gambia Government/Medical Research Council Joint Ethics Committee (proposal numbers: 670/630 and 1029v2).

Informed consent was obtained from parents or guardians of cases and mothers for the population controls (cord bloods). Population controls were collected by consenting women attending maternity units to give birth. Further controls were collected from The Gambia Biobank with appropriate ethics permissions.

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A case-control approach to the identification of polymorphisms associated with severe malaria in Kilifi, Kenya

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About this study

Malaria is a leading cause of death in much of sub-Saharan Africa (World Health Organization, 2008). Host genetic factors are thought to be important modifiers of severe and fatal malaria (Mackinnon MJ et al, 2005), yet little is known about which genes are involved or the degree of protection that they might confer.

Summary

This study was undertaken a case–control study of severe *P. falciparum* malaria in a rural area on the coast of Kenya to investigate the genetics of malaria resistance. Cases were children younger than 14 years who were admitted from within a defined study area to Kilifi District Hospital between June 1995 and February 2008. Controls consisted of children born consecutively within the same study area as cases who were recruited at 3-12 months of age into a genetic cohort study (the Kilifi Genetic Birth Cohort Study) between August 2006 and July 2008.

Clinical data and DNA samples were contributed to the MalariaGEN Consortial Project 1 (CP1) along with those of 11 other case-control studies from a total of 11 malaria-endemic countries. As part of the sample handling process, baseline genotyping data was generated for a number of malaria–associated single nucleotide polymorphisms (SNPs) and the appropriate data has been returned to each site for site-specific analysis. A total of 69 SNPs at candidate genes (selection based on previous reports of association with severe malaria or on their likely biological role in malaria infection/disease) will be included in our analysis and genome-wide SNP typing is planned for the future.

Single- and multi-locus analysis will be conducted using multivariate logistic regression, accounting for the effects of a range of confounders, to assess the relationship between genetic markers and well-defined severe malaria including sub-phenotypes such as severe malarial anaemia, cerebral malaria and respiratory distress.

Study site description

The KEMRI-Wellcome Trust Research Programme recruited severe malaria cases and healthy controls as part of on-going epidemiological studies of severe malaria at Kilifi District Hospital, Kenya. Kilifi District Hospital is situated in a rural area on the Kenyan Coast and has a catchment population of roughly of 500,000 people. The local economy is predominantly rural, being based on subsistence farming of maize, cassava, cashew nuts, and coconuts as well as dairy cows and goats. Two large agricultural estates, two research institutes and several tourist hotels contribute to local employment. The majority of the population fall within the Chonyi, Giriama and Kauma sub-divisions of the Mijikenda ethno-linguistic group.

Malaria transmission in Kilifi is seasonal, generally coinciding with the long and short rains in October and May, respectively. Recent years have seen a significant decline in the rate of transmission from meso-endemic in the 1990's to hypo-endemic transmission today. *P. falciparum* is the cause of the vast majority of malaria infections. Malaria is predominantly transmitted by mosquitoes of the species *Anopheles gambiae* (Mbogo CN et al, 1993). The overall annual entomological inoculation rate (EIR) has been estimated at 1-100 (Mbogo CN et al, 1993; Mbogo CM et al, 2003).

Methods

An unmatched case-control study was conducted in Kilifi District. Cases of severe malaria, resident within a defined study area, were recruited through a process of systematic surveillance of the paediatric wards at Kilifi District Hospital between June 1995 and February 2008. Cases consisted of children less than 14 years of age who were admitted to the high dependency ward with a primary diagnosis of severe malaria (defined by the presence of *P. falciparum* parasitaemia complicated by one or more of the following features: severe anaemia (Hb <5g/dl), convulsions before/during admission, cerebral malaria (Blantyre Coma Score ≤3) or respiratory distress).

Controls consisted of children born consecutively within the same study area as cases who are recruited at 3-12 months of age into a genetic cohort study (the Kilifi Genetic Birth Cohort Study) between August 2006 and July 2008.

A standardised case report form (CRF) was created for the Consortial Project and used by all sites to collect standardised clinical data. The data collected in Kenya (and all other sites) were uploaded onto secure web-based software developed by MalariaGEN. Here, the integrity of the data was checked and data was standardized and amalgamated.

Genomic DNA was extracted from whole blood, in Kilifi, using Qiagen DNeasy Blood kits (http://www.qiagen.com/) [Qiagen, Crawley, UK]. Aliquots of the DNA samples were shipped to the MalariaGEN Resource Centre in Oxford for further processing and quality control for quantity, quality (by genotyping) and confirming appropriate clinical data was available. Baseline genotype data for 69 malaria-associated SNPs was generated for all contributing samples; briefly, samples underwent a primer-extension pre-amplification (PEP) step (Xu K et al, 1993; Zhang L et al, 1992) prior to genotyping on the Sequenom® MassArray® platform. Following curation, the genotype data were returned to the PIs for local analyses.

Table 1: Breakdown of samples

Number	Gender: n (%)	Age in years: n (%)	Ethnicity: n (%)
Malaria cases: 2740	Male: 1358 (49) Female: 1235 (45) Not recorded: 175 (6)	<1: 526 (19) 1-2: 609 (22) 2-5: 1144 (42) 5-15: 297 (11) Not recorded: 1 (<1)	Chonyi: 621 (23) Giriama: 1622 (59) Kambe: 101 (4) Kauma: 197 (7) Other: 183 (7) Not recorded: 16 (<1)
Healthy controls: 4183	Male: 2058 (49) Female: 2022 (49) Not recorded: 103 (2)	<1: 4078 (97) 1-2: 105 (3)	Chonyi: 1508 (36) Giriama: 1930 (46) Kauma: 465 (11) Other: 273 (7)

Ethics

The study was approved by KEMRI Research Ethics Committee, Kilifi (proposal number: SCC1192).

Informed consent was obtained from parents or guardians of cases and mothers for the population controls. Population controls were collected by consenting women attending maternity units to give birth. DNA was extracted from cord blood samples.

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