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Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium falciparum* malaria

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Article summary

Article focus

- Genetic studies suggest a link between susceptibility to allergy and malaria in Africa
- We hypothesize that atopy increases susceptibility to malaria

Key messages

- Results demonstrate an association between asthma, atopic dermatitis and susceptibility to clinical *P. falciparum* episodes.
- Genetic pre-disposition to asthma or atopic dermatitis impairs the acquisition of clinical immunity to malaria.
- Administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Strengths and limitations

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association.

Abstract

Objectives: To assess the impact of atopy and allergy on the risk of clinical malaria.

Design: A clinical and immunological allergy cross-sectional survey in a birth cohort of 175 children from 1 month to 14 years of age followed for up to 15 years in a longitudinal open cohort study of malaria in Senegal. Malaria incidence data were available for 143 of these children (aged 4 months to 14 years of age) for up to 15 years. Mixed model regression analysis was used to determine the impact of allergy status on malaria incidence, adjusting for age, gender, sickle cell trait and force of infection.

Main outcome measures: Asthma, allergic rhinoconjunctivitis and atopic dermatitis status, the number of clinical *Plasmodium falciparum* malaria episodes since birth and associated parasite density.

Results: Twelve percent of the children were classified as asthmatic and ten percent as having atopic dermatitis. These groups had respectively a two-fold (OR 2.12 95% confidence intervals 1.46 to 3.08; P= 8 $x10^{-5}$) and three-fold (OR 3.15, 1.56 to 6.33; P= 1.3 $x10^{-3}$) increase in the risk of clinical *P. falciparum* malaria once older than the age of peak incidence of clinical malaria (3 to 4 years of age). They also presented with higher *P. falciparum* parasite densities (Asthma: mean 105.3 parasites/μL ±SE 41.0 *vs*. 51.3 ±9.7; P= 6.2 $x10^{-3}$; Atopic dermatitis: 135.4 ±70.7 *vs*. 52.3 ±11.0; P=0.014). There was no effect of allergy on the number of non-malaria clinical presentations. Individuals with allergic rhinoconjunctivitis did not have an increased risk of clinical malaria nor any difference in parasite densities.

Conclusion: These results demonstrate that asthma and atopic dermatitis delay the development of clinical immunity to *P. falciparum*. Despite the encouraging decrease in malaria incidence rates in Africa, a significant concern is the extent to which the increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines, administration to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Introduction

The World Allergy Organization estimates that 40% of the world's population is concerned by allergic diseases.[1] In developing countries where *Plasmodium falciparum* malaria is endemic, prevalence of allergy is significantly lower, but is on the increase.[2] T helper type 2 (Th2) cells, their related cytokines, IgE, eosinophils and mast cells play a major role in allergic inflammation. Orientation of the immune response towards a Th1 profile is crucial for immunity to intracellular pathogens,[3] whereas orientation towards a Th2 profile drives immunity to extracellular pathogens and antigens resulting in class switching giving rise to IgE-producing B cells.[4] A role of the Th1/Th2 balance in the development of clinical malaria following infection by *P. falciparum* has been suggested by numerous studies.[5-7] Whilst it is recognised that acquired anti-parasite immunity is IgG dependent,[8] parasite-specific IgE also impact upon the clinical outcome of infection. For example, higher IgE but not IgG levels have been observed in patients with cerebral malaria than those with uncomplicated P. falciparum infection.[9] The role of IgE, however, remains unclear. [10]

The interplay between infectious agents and allergy is ambiguous. On the one hand, for example, severe respiratory syncytial virus infection in infants increased the risk of allergic rhinoconjunctivitis and allergic asthma.[11,12] On the other hand, measles,[13] hepatitis A[14] and tuberculosis[15] seemingly reduce atopy. Although, an atopic condition can increase incidence of disease, such as the case for the skin commensal *Staphylococcus aureus* in patients with atopic dermatitis,[16] an atopic tendency *per se* does not generally lead to increased illness from infectious agents.

Genome wide studies have identified chromosomal regions linked to clinical malaria, all of which overlap with those previously identified to be involved in atopic dermatitis, asthma, atopy and IgE levels,[17-19] suggesting that common mechanisms may be involved in both pathologies.[20] Chromosomal region 5q31 that has been repeatedly shown to be associated with control of parasite density and contains a cluster of cytokines, among which IL12B has been previously associated with psoriasis.[21] The other regions, 13q13-q22, 5p15-p13 and 12q21-q23, contain genes involved in innate immunity, notably the interleukin 7 receptor, and several involved in tumour necrosis factor synthesis [C1q and tumour necrosis factor related protein 3 (C1QTNF3)] and a gene involved in the complement system (C9). [20]

Several additional lines of evidence support the concept that susceptibility to malaria and atopy may be related to similar immunological defects. In Ethiopia, a history of malaria was associated with atopy.[22] A mouse model for human atopic disease was found to be very susceptible to murine malaria and a major locus for atopic disease mapped close to the region controlling parasite density.[23] This region contains several candidate genes that have effects on T-cell function.[23]

Moreover, a direct effect of histamine in the malaria pathogenesis has been found using genetic and pharmacological approaches[24] and increased levels of histamine are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[25,26]

To test the hypothesis that allergy impacts upon clinical *P. falciparum* malaria, we performed a clinical allergy cross-sectional study in the family-based longitudinal cohort from Senegal previously used for the genome linkage study[20] and analysed the impact of asthma, atopic dermatitis, allergic rhinoconjunctivitis on the incidence of clinical *P. falciparum* episodes and the maximum parasite density during each episode.

Methods

Population and outcome data

The malaria research program conducted in Dielmo village in Senegal has been ongoing since 1990 as described elsewhere.[27] In brief, between 1990 and 2008, a longitudinal study involving the inhabitants of the village of Dielmo, Senegal, was carried out to identify all episodes of fever. The study design included daily medical surveillance with systematic blood testing of individuals with fever and examination of 200 oil-immersion fields on a thick blood film for malaria parasites (about 0.5 µL of blood). Each individual was given a unique identification code and details of family ties, occupation, and precise place of residence were recorded on detailed maps of each household with the location of each bedroom. All households were visited daily, absenteeism recorded, and the presence of fever or other symptoms assessed. We systematically recorded body temperature at home three times a week (every second day) in children younger than 5 years, and in older children and adults in cases of suspected fever or fever-related symptoms. In cases of fever or other symptoms,

blood testing was done at the dispensary by finger prick, and we provided detailed medical examination and specific treatment. Parasitologically confirmed clinical malaria episodes were treated according to national guidelines. From 1990 to 2008, four different drug regimens were implemented: Quinine from 1990 to 1994, Chloroquine from 1995 to 2003, Fansidar (sulfadoxine-pyrimethamine) from 2004 to mid-2006 and Artemisinin-based combination therapy (ACT; Amodiaquine- sulfadoxine-pyrimethamine) from mid-2006 to 2008.

Parasite positivity was established as follows. Thick blood films were prepared and stained by 3% Giemsa stain. Blood films were examined under an oil immersion objective at x1000 magnification by the trained laboratory technicians and 200 thick film fields were examined to count the number of asexual and gametocyte parasite stages. Asexual parasite densities (per µL) were calculated by establishing the ratio of parasites to white blood cells and then multiplying the parasite count by 8,000, the average white blood cell count per µL of blood.

Malaria transmission in Dielmo is intense and perennial. We conducted a cross-sectional survey to estimate the prevalence of symptoms related to allergic diseases among 175 children aged from 1 month to 14 years old who were born during the malaria research program.

Both the longitudinal and cross-sectional surveys were approved by the Ministry of Health of Senegal. Informed consent of the volunteers is renewed every year. More specifically for the cross-sectional survey, after informing about the procedures and the purpose of the study, written informed consent was obtained from parents or guardians of children either by signature or by thumbprint on a voluntary consent form written in both French and Wolof, the main local language. Consent was obtained in the presence of the school director, an independent witness.

The family structure (pedigree) was available after a demographic census performed for every volunteer at his adhesion in the project. A verbal interview of mothers or key representatives of the household was used to obtain information on genetic relationships between studied individuals, their children, their parents, and to identify genetic links among the population. The total pedigree comprised 828 individuals, including absent or dead relatives, composed of ten independent families that can be sub-divided into 206 nuclear families (father – mother couples with at least one child) with an average of 3.6

children each. Genetically related nuclear families occur because of multiple marriages and marriages among related individuals. Previous typing with microsatellites has enabled the construction of a pedigree based on Identity-by-Descent using MERLIN.[20,28] The mean coefficient of inbreeding is 0.0008. Newborns since this original genetic analysis were added to the family of the parents in question. The 143 children, with both allergy and malaria data, belonged to 61 nuclear families and comprised 30 singletons, 102 siblings and 11 half-sibs (yielding 55 half-sib pairs). The mean genetic relatedness (by pedigree) of the 143 children is 0.0114 (range: 0.0013 to 0.022).

P. falciparum *clinical episodes*

P. falciparum malaria clinical episode phenotypes analysed were: (i) clinical *P. falciparum* infections treated with anti-malarial therapy and (ii) the highest parasite density during the *P. falciparum* clinical episode. A clinical *P. falciparum* episode was defined as a clinical presentation with fever (axillary temperature ≥ 37.5°C) and/or other clinical signs suggestive of malaria associated with a thick blood smear positive for *P. falciparum* and that was treated with anti-malarial therapy. Repeated clinical malaria presentations within 15 consecutive days were not considered to be independent and were excluded from the analyses, unless there was a negative thick blood smear between two clinical presentations. We also excluded observations in any trimester for which the individual was not present for at least one third of the time.

We calculated the quarterly incidence rate of clinical *P. falciparum* episodes in children below the age of 15 years as the ratio of the total number of clinical *P. falciparum* episodes during the trimester divided by the total number of person-trimesters surveyed. Incidence rate is expressed as cases per 100 person-trimesters (see Supplementary Figure S1). This rate was used in the analysis to approximate the force of infection (exposure level) within the targeted population at the time of a given clinical *P. falciparum* episode.

The total number of clinical presentations per trimester that were not attributable to *P. falciparum* was tabulated. Repeated non-malaria presentations within seven consecutive days were not considered to be independent and were excluded.

Allergic diseases and atopic status

The International Study of Asthma and Allergies in Childhood (ISAAC) diagnostic criteria have been shown to be reproducible, adequate and able to discriminate children with allergic diseases in different areas of the world.[2] The standardized ISAAC questionnaire originally written in English was translated into French in compliance with ISAAC guidelines[29], adapting it to the usual local customs following advice from local clinicians and paediatric allergologists (Acknowledgements and Technical Appendix). The adequacy and reliability of the translated questionnaire had been previously confirmed by a pilot study on 30 randomly selected children in the same community. The questionnaire was completed by specially trained health workers during an oral interview conducted in Wolof with children and their mothers or guardians.

To assess the prevalence of allergic diseases in children, we used the positive and negative predictive values of the ISAAC questionnaire diagnosis criteria developed for subtropical countries.[30] Each question was scored according to the medical diagnosis of paediatricians and paediatric allergologists. Positive or negative answers were thus graded on the basis of symptom sensitivity, specificity, frequency, location or early onset. For each allergic disease, three categories of symptom severity, *severe*, *moderate*, and *none*, were defined as follows:

Asthma – severe symptoms if the child had "wheezing or whistling in the chest before the age of two years" and "more than three times" *or* severe enough to "limit his/her speech"; *moderate* symptoms if the child had "wheezing or whistling in the chest before the age of two years" and "in the past 12 months"; and *none* otherwise.

Allergic rhinoconjunctivitis – severe symptoms if the child had "sneezing, runny or stuffy nose in the past 12 months" and "more than five times a year", and "itchy, watery eyes or tropical endemic limboconjunctivitis (TELC) in the past 12 months"; *moderate* symptoms if the child had "sneezing, runny or stuffy nose in the past 12 months", and "itchy, watery eyes or TELC in the past 12 months"; and *none* otherwise.

Atopic dermatitis – severe symptoms if the child had "scaly or exudating, crusted and pruritic patches in the past 12 months" and "affecting any of the following characteristic areas: face, around the ears or eyes, folds of armpits or elbows or groin, behind the knees, under the buttocks", and "onset of symptoms before the age of two years"; *moderate* symptoms if the child had "scaly or exudating, crusted and pruritic patches in the past 12 months" and

"affecting any of characteristic areas (see above)", and "onset of symptoms before the age of four years"; and *none* otherwise.

The inter-relationships between variables reflecting the severity of symptoms of the three allergic diseases were used to identify children at high risk of atopy. The *high probability* group was defined by the prevalence of at least one of any *severe* symptoms or two of any *moderate* symptoms. The *probable* group was defined as those with *moderate* symptoms from one of the three allergic diseases and remaining children were classified in the *unlikely* group.

Helminths

Helminthic infections are common in this region and are known to modify the clinical course and outcome of both allergic diseases and malaria.[31,32] We therefore carried out a helminth survey for 91 individuals present during the cross-sectional survey. Diagnosis was performed by stool examination by microscope and by the Kato technique to search for the presence of *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*), whipworm (*Trichuris trichiuria*), *Schistosoma mansoni*, and *Strongyloides stercoralis*. Examination for pinworms (*Enterobius vermicularis*) was performed by the anal scotch-test. An anti-helminthic treatment was proposed for all infested individuals.

Immunoglobulin E titres

Specific IgE titres were measured by ELISA as previously described.[33] A panel of allergens of potential pertinence to the three classes of allergy was used: (i) Salivary gland extracts (SGE) of two mosquito species present in the study cohorts, *Aedes aegypti* and *Anopheles gambiae sensu stricto*, and (ii) *P. falciparum* parasite extract were prepared as previously described[31]; (iii) House dust mite spp. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*; (iv) a mix of pollen allergens from five ubiquitous graminae spp. [Cock's-foot (*Dactylis glomerata*), Timothy grass (*Phleum pratense*), Sweet Vernal grass (*Anthoxanthum odoratum*), Perennial ryegrass (*Lolium perenne*), Kentucky Bluegrass (*Poa pratensis*)] (all from Stallergenes, France).

Statistical analysis

Statistical analyses were performed using R version 2.12.0 (The R Foundation for Statistical Computing, Vienna, Austria). To address the effect of allergic status on the risk of clinical *P.*

falciparum episodes, we performed Generalized Linear Mixed Models (GLMM) extended to pedigree data using the *pedigreemm* package for R to account for the non-independence of individuals because of family relationships, shared house and for repeated measures from the same individual (Technical Appendix). Correlated individual effects due to familial relationships were taken into account by using the pedigree-based genetic relatedness matrix that contains the genetic covariance among all pairs of individuals in the study cohort and is calculated using the pedigree information.[34] Shared house and repeated measures from the same individual were modelled as random effects. All random effects were assumed to be normally distributed, and conditional on these random effects, the dependent variable had: (i) a Binomial distribution when the studied phenotype was the occurrence of a clinical *P. falciparum* episode treated with anti-malarial therapy during a trimester, (ii) a Gaussian distribution when the studied phenotype was the logarithm of the maximum parasite density during a given clinical *P. falciparum* episode, and (iii) a Poisson distribution when the studied phenotype was the number of non-malaria episodes per trimester. The effects of allergy disease classes on these dependent variables were modelled as fixed effects. Allergy classes were reduced to two levels, *Severe* or *moderate vs*. *none* for analyses of asthma, atopic dermatitis and allergic rhinoconjunctivitis and *high probability vs*. *probable* and *unlikely* for atopic tendency. Co-variables included sickle cell trait[33], gender, number of days present on site during the trimester, trimestrial incidence of *P. falciparum* and age. Age was initially analysed as a continuous covariate. To assess the age-specific effect of allergy, age was categorised into two levels (<3.5 years of age and ≥3.5 years of age, based on the age of peak clinical incidence) and allergy class was nested within age class. The age threshold was varied from 1.5 years to 5.5 years of age and the data re-analysed to assess at which age there was the strongest effect. The association of allergy classes with IgE levels was analysed by box-cox transforming the data and fitting a GLMM with a normal distribution.

Results

Of the 205 eligible children aged under 15 years involved in the family-based longitudinal study, 175 (85.4 %) participated in the cross-sectional survey to assess the prevalence of related symptoms of allergic diseases. All eligible children present at the time of the survey

were included; no explicit refusal to participate was recorded. The study cohort was aged from 1 month to 14 years 11 months. The sex-ratio (male/female) was 0.94.

From 1994 until 2008, 143 of the children participating in the cross-sectional survey were present for at least 31 days in any trimester during the study period generating a total of 3,093 person-trimesters of presence (Supplementary Table S1). There were 2,065 treated *P. falciparum* clinical episodes (per individual: median 11, range 0-47)(Supplementary Table S2). The age peak of incidence of *P. falciparum* episodes occurred at 3 to 4 years of age (Figure 1). There were 1,868 non-malaria episodes (median 12, range 0-37) (Table S2). These non-malaria clinical presentations were associated with headache (38 %), chills (32 %), cough (13 %), vomiting (11 %) and diarrhoea (6 %).

The prevalence of moderate or severe asthma symptoms was respectively 2.3 % and 10.3 % (Table 1). The prevalence of moderate or severe allergic rhinoconjunctivitis symptoms was respectively 6.3 % and 10.3 %. The prevalence of moderate or severe atopic dermatitis symptoms was respectively 6.3 % and 2.9 %. On the basis of symptom severity, an atopic tendency was estimated to be unlikely for 68.0 %, probable for 9.1 % and highly probable for 22.9 % of the 175 children. The frequency of each allergy class in children for whom malaria data were available is shown in Table S1.

The risk of treated clinical *P. falciparum* infections was higher for children with high probability of atopy (OR 1.65, 95% confidence intervals 1.20 to 2.26; P=0.002) (Table 2), after adjusting for age, sickle cell trait and the exposure level. Gender was not found to be significant. Analysing the impact of atopy in children younger and older than the peak age of clinical incidence (3 to 4 years old), revealed that atopy increased the risk of *P. falciparum* episodes in children at an age greater than 3.5 years (OR 2.02, 1.39 to 2.93; $P=2x10^{-4}$), but not in children of age prior to the peak clinical incidence (OR 1.38, 0.92 to 2.08; P=0.124) (Table 2). This increased risk resulted in an ever increasing cumulative number of *P. falciparum* episodes with age beyond that of peak clinical incidence (Figure 2. See supplementary Figure S2 for model predictions for comparison).

Analysis by allergy category revealed that asthma (severe or moderate) increases the risk of *P. falciparum* episodes (OR 2.12, 1.46 to 3.08; $P= 8 x10^{-5}$) and this again only in children of age greater than 3.5 years old (OR 2.33, 1.50 to 3.61; $P= 1.5 x10^{-4}$). Atopic dermatitis increased the risk of clinical malaria in children older (OR 3.15, 1.56 to 6.33; $P= 1.3 x10^{-3}$) but

not younger than 3.5 years of age (Table 2). Allergic rhinoconjunctivitis was not associated with increased risk of clinical malaria at any age (Table 2). The impact of atopy, asthma and atopic dermatitis can be clearly seen in the ever-increasing number of cumulative *P. falciparum* episodes beyond the age of the onset of clinical immunity in the population, 3.5 years of age (Figure 2). There is no difference in the number of clinical malaria episodes prior to this age in individuals with or without an allergic condition. Analysis using different age thresholds (from 1.5 to 5.5 years of age) revealed similar OR for thresholds of 2.5, 3.5 and 4.5 years of age. The maximum OR for increased malaria occurred in children older than 4.5 years of age and with atopy or atopic dermatitis, whereas for the asthma group it occurred in children after 3.5 years of age (Supplementary Table S3).

There was no impact of any allergic disease on the number of non-malaria episodes by trimester (Supplementary Table S4).

The impact of atopy, asthma and atopic dermatitis on the maximum *P. falciparum* parasite density during a given clinical malaria episode mirrored that of the risk of *P. falciparum* episodes. Parasite density was significantly higher for children with allergic disease older than 3.5 years of age (Table 3 and supplementary Figure S3 for residuals of the fitted model). As the log-transformed data were left skewed, we additionally analysed using box-cox transformation and probit normalization of the data. The results were qualitatively the same (Supplementary text and Figures S4-S8). Allergic rhinoconjunctivitis had no impact on the parasite density (Table 3). Analysis using different age thresholds yielded similar qualitative conclusions as seen with the number of clinical episodes (Table S3).

Individuals with moderate or severe symptoms of atopic dermatitis had significantly higher specific IgE titres against *Ae. aegypti* (P=0.004) and *An. gambiae* SGE (P<0.001). There were no detectable specific anti-*P. falciparum* IgE. Individuals with moderate or severe symptoms of allergic rhinoconjunctivitis did not have significantly higher IgE titres against the tested graminae (P=0.28), although titres decreased with age (P=0.035). There was also no effect of asthma on IgE titres against the house dust mite spp. tested (*D. farinae* P=0.60 & *D. pteronyssinus* P=0.27).

Only five individuals were infested with helminths (two *Ancylostoma*, one *Strongyloides*, one *Trichuris* and one *Enterobius*).

Discussion

Principal findings

Establishing the allergic status of children up to the age of 15 years old followed for malaria since birth, revealed an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

Strengths and weaknesses of the study

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association. In addition, although allergy diagnosis for children under 2 years of age is not considered reliable, there were only 15 individuals under 2 at the time of the allergy study of the 143 for whom malaria and allergy data were available.

Meaning of the study

Under intense malaria transmission, after repeated exposure to the parasite, children develop a clinical immunity[35], whereby they tolerate elevated parasite densities without showing clinical symptoms. In this cohort, the population mean onset of clinical immunity occurred at 3 to 4 years of age. Although clinical immunity is accompanied by a reduction in parasite density, effective anti-parasite immunity develops much more slowly[36] with individuals achieving a state of premunition, whereby they maintain low-grade parasite densities in an asymptomatic state.[37] We show here that children with clinically defined asthma or atopic dermatitis have an increased risk of presenting with *P. falciparum* malaria episodes requiring treatment once passing the age of peak clinical incidence. They also had higher parasite density during clinical episodes, suggesting a reduced ability to control parasite replication. The observed increase in clinical incidence of malaria in patients with asthma or atopic dermatitis is not likely to be the result of increased frailty of such individuals; these individuals did not come more frequently to the clinic with non-malaria

symptoms. Our previous genome linkage study identifying chromosomal regions[20] associated with malaria that overlap with those previously shown to be linked to asthma/atopy suggests that there may be a shared genetic basis to these pathologies rather than any causative effect of one on the other. This is consistent with the increased susceptibility to malaria of mouse atopic models.[23]

Comparison with other studies

A previous study in Ethiopia (East Africa) found that a history of malaria (yes/no) increased risk of atopic dermatitis in 306 cases compared to 426 controls as characterized using the ISAAC questionnaire.[22] The only other epidemiological study that has previously examined the link between malaria and atopy[38] also interpreted the result from the perspective of the impact of malaria on atopy. They examined the re-infection rate with *P. falciparum* over a 5-year period in 91 children that were subsequently classified as atopic or not using skin prick tests (SPT) with house dust mite antigen. Their conclusion was that, as with measles[13] and tuberculosis[15], malaria infection reduces atopy. However, the study lacked previous infection data since birth of the participating individuals and focussed on atopy as determined by SPT against a single allergen. The case-control study of atopic dermatitis risk factors cited above found no overall association between allergen skin sensitization and atopic dermatitis. We also found no evidence of increased IgE titres against house dust mites in the asthmatic or atopic dermatitis groups or against grass pollen in individuals with allergic rhinoconjunctivitis. Such differences likely reflect the different IgE reactivity profiles due to differences in allergen exposure in Africa.[39] There was no evidence of anti-parasite IgE in this cohort of children. We previously showed that circulating anti-parasite IgE titres were strongly positively correlated with anti-mosquito saliva IgE, but became undetectable following malaria exposure, potentially being bound to effector cells.[33] Only mosquito saliva, a known major local allergen, induced a specific IgE response at significantly higher titres in individuals with atopic dermatitis.

Although the immune effectors of clinical immunity are still poorly defined, there is strong evidence that acquired anti-parasite immunity is IgG-dependent[8] and cytophilic immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by opsonisation and/or by Antibody Dependent Cellular Immunity play an important role in premunition.[37] The higher parasite density during symptomatic episodes observed in the

asthma group suggests impaired development of acquired immunity. Impaired acquisition of immunity to malaria in children with asthma or atopic dermatitis may stem from their imbalanced Th1/Th2 response. Indeed, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*. Dendritic cells that are oriented to a Th2 phenotype are more susceptible to orient the acquired immune response towards a Th2 profile.[40] Orientation of the immune response towards a Th2 profile by asthma or atopic dermatitis would result in a poor Th1 response (and hence development of protective IgG immunoglobulins), considered to be the dominant arm of the immune response enabling resistance to infectious disease in children.[41]

Many studies have revealed an important role of histamine, a key downstream effector molecule in allergic reaction, in the outcome of a malaria parasite infection.[24-26,42-45] Moreover, reports indicate that components of the innate immune system, including eosinophils, basophils, and mast cells (MCs), could play important roles in the pathogenesis of malaria.[42] Increased levels of histamine in plasma and tissue, derived from basophils and MCs, notably following stimulation by IgE through the high affinity receptor FcεR1, are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[25,26] Chlorpheniramine, a HR1agonist reversed resistance to chloroquine and amodiaquine both *in vivo* and *in vitro*.[43] Moreover, astemizole, another HR1 agonist, was identified as an anti-malarial agent in a clinical drug library screen.[44] Finally, *P. falciparum* produces translationally controlled tumor protein, which is a homolog of the mammalian histamine-releasing factor that causes histamine release from human basophils.[45]

Further research

Our results provide the first birth cohort study addressing the link between malaria and allergic diseases. They contribute to a growing body of evidence that the pathologies are related. ISAAC has revealed a steady but significant increase in prevalence rates of asthma and allergic diseases in Africa. Whilst the majority of studies have focused on large cities, there is increasing urbanization throughout Africa, as well as improved access to primary health care in many areas. A key concern for ISAAC is the extent to which such societal evolution will result in an increase in allergic diseases. Increased urbanization in sub-Saharan Africa is changing the epidemiology of malaria and although resulting in a decrease in risk, will result in more severe clinical malaria in older individuals.[46,47] Moreover, a large

consumption of anti-malarial drugs in the urban areas provides substantial drug pressure fostering, the selection of drug-resistant parasites. Despite the encouraging recent decrease in malaria incidence rates, even in rural areas, an additional significant concern is the extent to which such an increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines,[48] administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials[49] and alleviate the non-infectious consequences of atopy. Clinical intervention studies should be envisaged.

What is already known on this topic

There are several reports of the beneficial effects of anti-histamines for malaria chemoprophylaxis[24-26,48] as well as our previous work[20] showing that chromosomal regions associated with malaria are also linked to allergy and atopy.[17-19] There are two epidemiological studies showing opposite effects of malaria on atopy.[22,38]

What this study adds

Using a longitudinal malaria study birth cohort, we identified an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred only after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: The allergy study was approved by the Senegalese National Ethics committee (2009/N°46). Renewed approval of the longitudinal malaria study was obtained from the same committee (2006/N°969).

Data sharing: The allergy database will be made available on-line. The longitudinal malaria data set will be made available following discussion with the coordinators of the three Institutes that govern the dataset through contact with the corresponding author.

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Table 1 Classification of Asthma, Allergic rhinoconjunctivitis, Atopic dermatitis and overall Atopic status according to ISAAC questionnaire in children aged 0-14 from a malaria birth cohort. N is total number of children examined and n-malaria represents those for whom malaria data were recorded. F is the number of females and M the number of males.

	N (F/M)	%	n-malaria (F/M)
Asthma symptoms			
None	153 (73/80)	87.43	125 (59/66)
Moderate	4 (1/3)	2.29	4 (1/3)
Severe	18 (6/12)	10.29	14 4/10)
Rhinoconjunctivitis symptoms			
None	146 (64/82)	83.43	120 (52/68)
Moderate	11 (8/3)	6.29	9(6/3)
Severe	18 (6/12)	10.29	14 (6/8)
Atopic dermatitis symptoms			
None	159 (75/84)	90.86	128 (60/68)
Moderate	11 (1/10)	6.29	11 (1/10)
Severe	5 (4/1)	2.86	4 (3/1)
Atopic tendency			
Unlikely	119 (56/63)	68.00	97 (46/51)
Probable	16 (8/8)	9.14	14 (6/8)
Highly probable	40 (16/24)	22.86	32 (12/20)

Table 2 Impact of allergy status on risk of *P. falciparum* clinical episodes. Shown are the *P* values and adjusted Odds Ratios with 95% confidence intervals calculated from the mixed model analyses. Values for the covariables Age (≥3.5 years of age compared to <3.5 years of age), Trimestrial incidence of *P. falciparum* clinical episodes and HbAS (beta-globin sickle cell trait; AS compared to AA) are those from the Asthma model analysis. For clarity significant co-variables are shown in bold.

	Age groups <3.5 years>	ORa	95% Confidence Intervals		*P* value
			Lower	Upper	
Atopy	**Both**	**1.65**	**1.20**	**2.26**	**2.0×10^{-3}**
	<3.5	1.38	0.92	2.08	0.124
	≥3.5	**2.02**	**1.39**	**2.93**	**2.1×10^{-4}**
Asthma	**Both**	**2.12**	**1.46**	**3.08**	**8.0×10^{-5}**
	<3.5	1.50	0.90	2.50	0.122
	≥3.5	**2.33**	**1.50**	**3.61**	**1.5×10^{-4}**
Atopic dermatitis	Both	1.05	0.65	1.70	0.842
	<3.5	0.84	0.49	1.46	0.539
	≥3.5	**3.15**	**1.56**	**6.33**	**1.3×10^{-3}**
Rhinoconjunctivitis	Both	0.96	0.65	1.41	0.818
	<3.5	1.05	0.64	1.72	0.853
	≥3.5	0.95	0.60	1.52	0.834
Age ≥3.5		**0.48**	**0.40**	**0.57**	**2.7×10^{-15}**
Trimestrial incidence		**1.01**	**1.00**	**1.01**	**1.8×10^{-6}**
HbAS		**0.24**	**0.12**	**0.47**	**3.7×10^{-5}**

Table 3 Impact of allergy status on the maximum *P. falciparum* parasite density during a clinical malaria episode. Shown are the back-transformed mean parasite densities per microlitre and standard errors (SEM) estimated from the GLMM analyses after taking into account the other co-variables. Significantly different effects are shown in bold for clarity.

Allergic condition	Age groups	Allergic status (No/Yes)	Mean parasite density	SEM	*P* value
Atopy	**Both**	**N**	**76.3**	13.8	
		Y	**131.0**	36.4	**0.0158**
	<3.5	N	114.3	23.7	
		Y	171.1	56.0	0.148
	≥3.5	**N**	**48.4**	9.8	
		Y	**114.8**	37.1	**$9.5x10^{-4}$**
Asthma	**Both**	**N**	**78.1**	14.4	
		Y	**148.5**	44.3	**$3.8\ x10^{-3}$**
	<3.5	N	114.8	24.3	
		Y	171.9	74.5	0.167
	≥3.5	**N**	**51.3**	9.7	
		Y	**105.3**	41.0	**$6.2\ x10^{-3}$**
Atopic dermatitis	Both	N	82.6	15.0	
		Y	93.9	38.9	0.605
	<3.5	N	122.6	25.5	
		Y	133.9	63.5	0.425
	≥3.5	**N**	**52.3**	11.0	
		Y	**135.4**	70.7	**0.014**
Rhinoconjunctivitis	Both	N	81.5	14.8	
		Y	111.4	39.0	0.570
	<3.5	N	118.8	25.1	
		Y	166.3	69.9	0.537
	≥3.5	N	54.6	11.3	
		Y	80.9	33.7	0.327

Figure 1 Annual incidence rate of clinical *P. falciparum* episodes per 100 children (bold line). In order to overcome the fluctuations of the annual incidence rate, we fit second (dotted line), third (dashed line) and fourth (solid line) degree polynomial trend lines to the data (bold line). The corresponding R-squared values are 0.70, 0.91 and 0·99 respectively indicating an accurate fit for third and fourth order polynomials. The inflexion on these two trend lines indicates the onset of acquisition of clinical immunity at approximately 3 to 4 years of age.

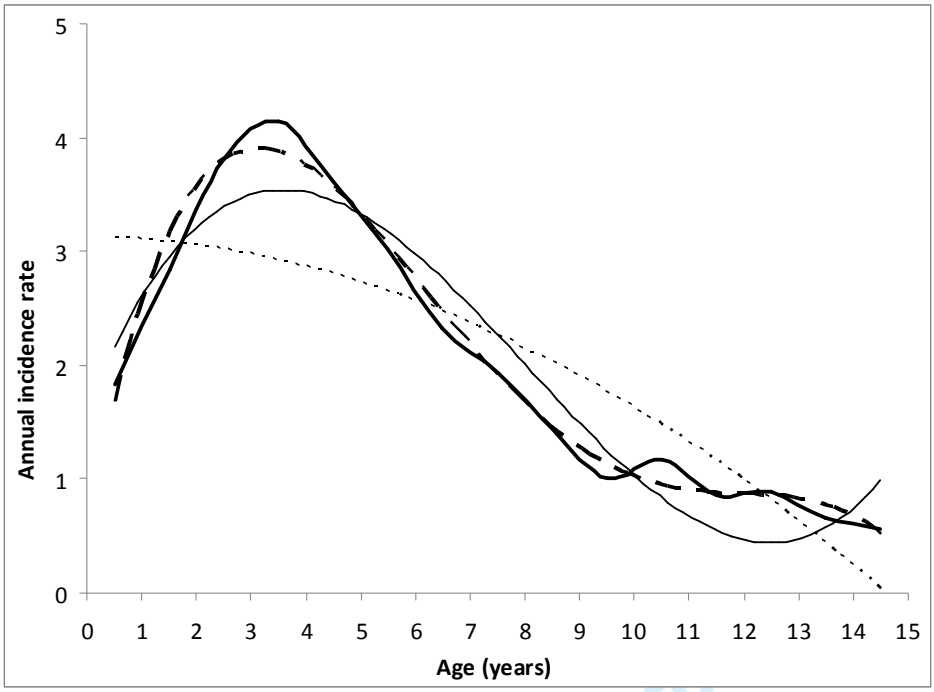
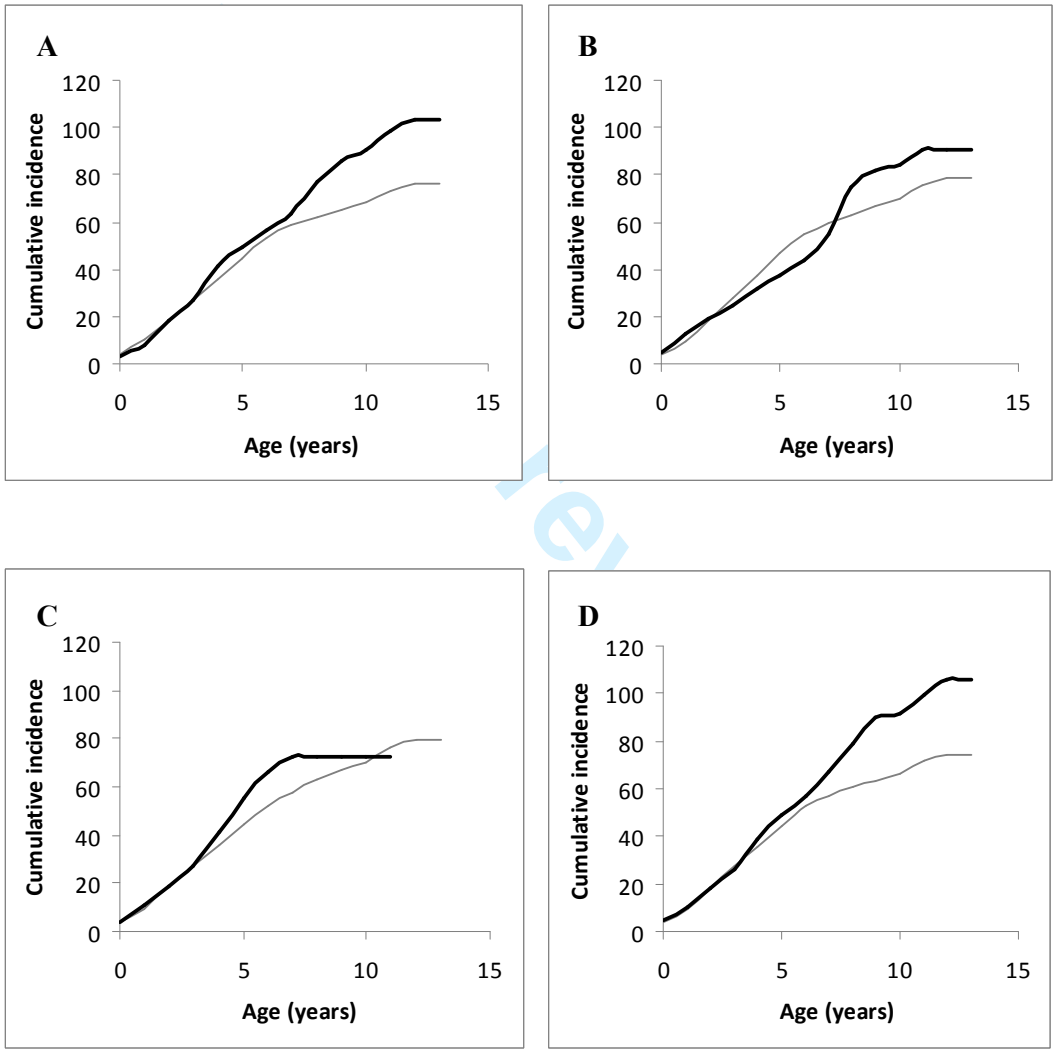


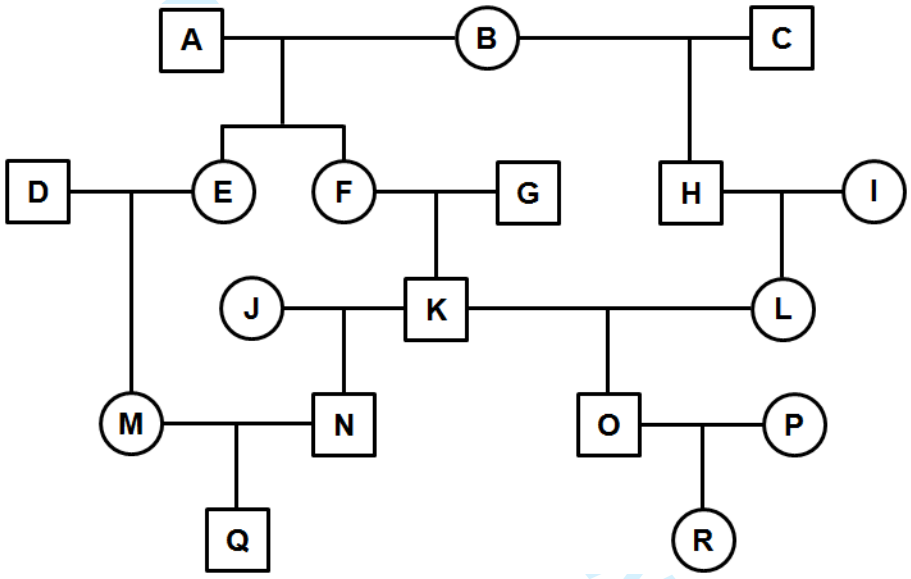
Figure 2 Mean cumulative number of *P. falciparum* clinical episodes with age for the (A) Asthma, (B) Rhinoconjunctivitis and (C) Atopic dermatitis classes and overall Atopy class (D) (bold lines) compared to individuals without symptoms of each respective allergy type (thin lines). In all cases moderate and severe classes are combined and compared to individuals without allergy symptoms. Note there are no children older than 11 years of age with Atopic dermatitis.



Pedigree-based genetic relatedness

The Genetic covariance between two individuals can be computed using the pedigree information. For individuals A and B, a given pair in a pedigree, the genetic covariance is computed as $r(A,B) = 2\times coancestry(A,B)$ where the *coancestry* between A and B is calculated referring to the method presented by Falconer and Mackay in 1996 (Falconer and Mackay 1996): $coancestry(A,B) = \Sigma_p(1/2)^{n(p)}\times(1 + I_{Common\ Ancestor})$ where p is the number of paths in the pedigree linking A and B, $n(p)$ the number of individuals (including A and B) for each path p and I_X is the *inbreeding* coefficient of X also equal to the *coancestry* between the two parents of X, I_X is set to 0 if X is a founder.

Illustration: Consider, as an example, the pedigree below containing 18 individuals named {A, B, …, R} for the calculation of genetic covariance's.



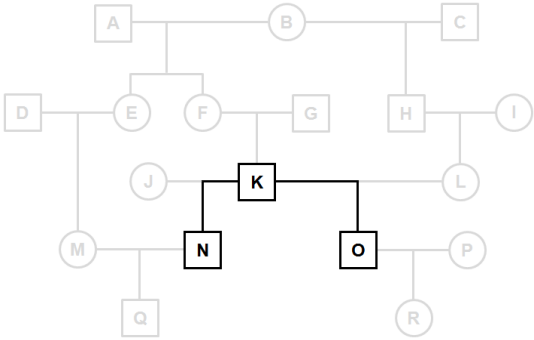
Pedigree structure.

The genetic relatedness between individuals N and O is equal to 0.266. This value is calculated as followed:
The number of paths linking N and O from the pedigree structure above is $p = 2$.
As illustrated below:

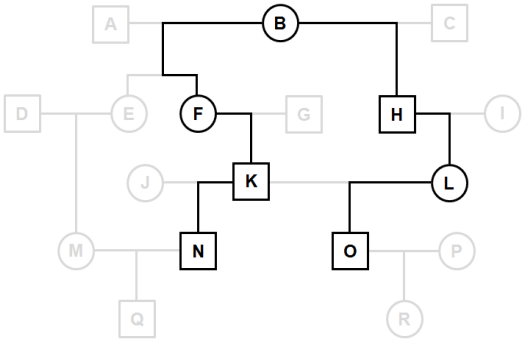
- **Path 1** contains $n(1) = 3$ individuals {N, K, O} with K as the common ancestor. Inbreeding coefficient of K, I_K , is the *coancestry* between the two parents of K (F and G) and is null because F and G are not genetically linked.
- **Path 2** contains $n(2) = 7$ individuals {N, K, F, B, H, L, O} with B as the common ancestor. Inbreeding coefficient of B, I_B , is null because B is a founder.

Therefore, genetic relatedness between individuals N and O is:

$$= 2\times(0.5^{n(1)}\times(1+I_K) + 0.5^{n(2)}\times(1+ I_B))$$
$$= 2\times(0.5^{3}\times(1+0) + 0.5^{7}\times(1+0)) = 0.266$$



Path 1 linking N and O.



Path 2 linking N and O.

Defining an equivalent model design where individual effects are independent using the genetic relatedness matrix:

Let us rename $Y^* = l(\mu)$. Y^* can be consider as a linearization of the phenotype through the link function l. The expected mean of Y^* and the variance of Y^* are:

(i) $E(Y^*) = E(X\beta + Z\gamma + \varepsilon)$
$= E(X\beta) + E(Z\gamma) + E(\varepsilon) = X \times E(\beta) + Z \times E(\gamma) + E(\varepsilon)$
$= X\beta$ (asymptomatically).

(ii) $Var(Y^*) = Var(X\beta + Z\gamma + \varepsilon)$
$= Var(Z\gamma + \varepsilon)$ (as $X\beta$ is the fixed part, thus has variance equal to 0)
$= Var(Z\gamma) + Var(\varepsilon)$ (as γ and ε are independent)
$= Z \times Var(\gamma) \times Z^T + Var(\varepsilon)$ (Z^T is the transpose of Z)
$= Z(A\sigma_g^2)Z^T + I\sigma_r^2$
$= ZAZ^T\sigma_g^2 + I\sigma_r^2$

If individuals were independent, i.e. $A = I_N$, variance of Y^* could be expressed as $ZZ^T\sigma_g^2 + I\sigma_r^2$. However, using linear algebra theory by the method "Cholesky decomposition of a matrix", we can show that there is an equivalent expression of the variance of Y^* corresponding to the modeling of data from independent individuals, having γ^* as an equivalent vector of random effects and Z^* an equivalent design matrix relating γ^* to Y^* so that:
$Var(Y^*) = Z^*(I\sigma_g^2)Z^{*T} + I\sigma_r^2$. $I\sigma_g^2$ is then the covariance matrix of the equivalent independent random individual effects γ^*.

Theorem: *Cholesky decomposition of a matrix*
If A is a symmetric positive-definite matrix, there is a triangular matrix L so that A can be written as $A = LL^T$. L can be seen as the "square root" of the matrix A.
Note that the genetic relatedness matrix A computed using the pedigree information (Falconer and Mackay 1996) is a positive-definite matrix, unless identical twins are in the pedigree in which case it would be positive semi-definite.

Equivalent model with independent random effects: We set $A = LL^T$ then:
$Var(Y^*) = Z(A\sigma_g^2)Z^T + I\sigma_r^2$
$= Z(LL^T\sigma_g^2)Z^T + I\sigma_r^2$

$= ZLL^{T}Z^{T}\sigma_g^2 + I\sigma_r^2$
$= (ZL)(ZL)^{T}\sigma_g^2 + I\sigma_r^2$
$= (Z^*)(Z^*)^{T}\sigma_g^2 + I\sigma_r^2$ (where we set $Z^* = ZL$)

Then, if we define $\gamma^* = L^{-1}\gamma$, we can rewrite the model as:

$Y^* = X\beta + Z^*\gamma^* + \varepsilon$ (because $Z\gamma = Z(LL^{-1})\gamma = (ZL)(L^{-1}\gamma) = Z^*\gamma^*$),

and the γ_i^* are independent, in other terms $Var(\gamma^*) = I\sigma_g^2$, as demonstrated below:

We assumed that $\gamma \sim N(0, A\sigma_g^2)$. Then $\gamma^* = L^{-1}\gamma$ is also distributed as a multivariate Normal with mean $E(\gamma^*) = L^{-1}E(\gamma) = L^{-1}\times 0 = 0$ and variance:

$Var(\gamma^*) = (L^{-1})\times Var(\gamma)\times(L^{-1})^{T}$
$= (L^{-1})\times A\sigma_g^2\times(L^{-1})^{T} = (L^{-1})LL^{T}(L^{-1})^{T}\sigma_g^2$
$= (L^{-1}L)(L^{-1}L)^{T}\sigma_g^2$
$= I\sigma_g^2$

The random effects are now independent and then the classical mixed model assuming independence between levels (here individuals) is applied, and the estimate of fixed effects obtained are fine, i.e. corrected for genetic relationships.

References

Falconer DS, Mackay TFC (1996) Introduction to Quantitative Genetics. 4th Edn. London: Longman.

Supplementary Tables

Table S1 Number of person-trimesters contributed by number of children by age class and the number who had severe/moderate allergy symptoms, for whom malaria data were also available. AS – Asthma, AD – Atopic dermatitis, RC – Rhinoconjunctivitis. Shown also are the numbers of these individuals suffering from two or all three allergy conditions.

Age group	N° person-trimesters	N° people	AS	AD	RC	AS+AD	AS+RC	AD+RC	AS+AD+RC
]1	7	6	1	2	2	0	1	0	0
]2	21	9	0	1	3	0	0	0	0
]3	48	11	1	1	2	0	0	1	0
]4	119	12	1	2	3	0	0	1	0
]5	102	11	3	4	3	2	1	2	1
]6	125	11	1	1	0	0	0	0	0
]7	303	11	1	2	1	1	0	0	0
]8	340	12	1	1	1	1	0	0	0
]9	362	10	2	0	1	0	1	0	0
]10	610	17	1	0	3	0	0	0	0
]11	77	4	2	1	0	0	0	0	0
]12	484	16	3	0	3	0	1	0	0
]13	390	10	1	0	0	0	0	0	0
]14	105	3	0	0	1	0	0	0	0
Total	3093	143	18	15	23	4	4	4	1

Table S2 Summary of total number of person-trimesters with non-malaria and symptomatic *P. falciparum* clinical presentations and total number of non-malaria episodes according to age class. Given are the number of people contributing to each type of presentation.

	Age group (years)	
	<3·5	**≥3·5**
Total person-trimesters	1283	1810
People	126	113
Total *P. falciparum* symptomatic trimesters	963	1102
People	114	108
Total non-malaria episodes	754	1114
People	123	109

Table S3 Effect of changing age threshold on impact of allergy on the risk of clinical malaria and concomitant parasite density. Given are Odds Ratio with 95% confidence intervals, for clinical malaria episodes and the beta coefficient and standard error for parasite density. Corresponding P values are also given. Values are from the nested GLMM analyses.

A. Malaria episodes							**B. Parasite density**				
Age cut-off (years)	OR	95% CI	P value	OR	95% CI	P value	Age cut-off	beta coeff (se)	P value	beta coeff (se)	P value
Atopy		above threshold			below threshold		Atopy	above threshold		below threshold	
1.5	1.80	1.25-2.59	1.7x10-3	1.57	0.85-2.89	0.15	1.5	0.70 (0.27)	9.2x10-3	0.54 (0.35)	0.12
2.5	2.00	1.39-2.88	2.0x10-4	1.23	0.76-1.99	0.40	2.5	0.79 (0.26)	2.6x10-3	0.35 (0.29)	0.23
3.5	2.02	1.39-2.93	2.1x10-4	1.38	0.92-2.08	0.12	3.5	0.85 (0.26)	9.5x10-4	0.37 (0.26)	0.15
4.5	2.10	1.42-3.10	1.6x10-4	1.41	0.98-2.04	0.063	4.5	0.87 (0.25)	6.9x10-4	0.40 (0.23)	0.09
5.5	1.64	1.07-2.52	0.02	1.67	1.17-2.37	0.004	5.5	0.73 (0.27)	7.4x10-3	0.48 (0.22)	3.4x10-3
Asthma							Asthma				
1.5	1.98	1.29-3.03	1.8x10-3	1.46	0.69-3.19	0.34	1.5	0.66 (0.31)	0.03	0.30 (0.44)	0.48
2.5	2.30	1.49-3.55	1.6x10-4	1.15	0.63-2.09	0.65	2.5	0.78 (0.30)	0.01	0.26 (0.36)	0.48
3.5	2.33	1.50-3.61	1.5x10-4	1.50	0.90-2.50	0.12	3.5	0.82 (0.30)	6.2x10-3	0.43 (0.31)	0.17
4.5	2.30	1.48-3.59	2.4x10-4	1.76	1.11-2.80	0.017	4.5	0.81 (0.29)	5.8x10-3	0.56 (0.28)	0.049
5.5	1.98	1.22-3.22	0.006	2.06	1.33-3.18	0.0011	5.5	0.72 (0.31)	0.02	0.62 (0.27)	0.02
Atopic Dermatitis							Atopic Dermatitis				
1.5	2.05	1.18-3.56	0.01	0.91	0.42-1.97	0.80	1.5	0.80 (0.37)	0.03	0.72 (0.46)	0.12
2.5	2.49	1.36-4.57	3.1x10-3	0.82	0.44-1.53	0.53	2.5	0.77 (0.38)	0.044	0.52 (0.39)	0.19
3.5	3.15	1.56-6.33	1.3x10-3	0.84	0.49-1.46	0.54	3.5	0.99 (0.40)	0.014	0.28 (0.35)	0.42
4.5	3.79	1.61-8.92	2.3x10-3	0.94	0.57-1.57	0.82	4.5	0.98 (0.47)	0.036	0.29 (0.32)	0.37
5.5	1.33	0.47-3.77	0.59	1.19	0.73-1.96	0.49	5.5	0.26 (0.61)	0.67	0.38 (0.31)	0.22
Rhinoconjunctivitis							Rhinoconjunctivitis				
1.5	1.04	0.66-1.62	0.88	1.01	0.51-2.01	0.98	1.5	0.36 (0.32)	0.27	0.18 (0.41)	0.66
2.5	1.01	0.64-1.61	0.96	0.96	0.55-1.68	0.89	2.5	0.28 (0.33)	0.40	0.25 (0.35)	0.48
3.5	0.95	0.60-1.52	0.83	1.05	0.64-1.72	0.85	3.5	0.31 (0.32)	0.33	0.19 (0.31)	0.54
4.5	0.87	0.54-1.42	0.59	1.06	0.68-1.66	0.79	4.5	0.20 (0.32)	0.53	0.22 (0.28)	0.44
5.5	0.81	0.48-1.36	0.43	1.07	0.70-1.64	0.74	5.5	0.10 (0.33)	0.75	0.23 (0.27)	0.39

Table S4 Frequency of non-malaria episodes (number of days of presence divided by number of non-malaria episodes) according to allergic status and age group. The *P* value is that from the GLMM analyses of the effect of allergic status by age group on the number of non-malaria episodes per person-trimester.

Allergic condition	Allergic status (No/Yes)	Age group (years) <3·5	Age group (years) >3·5	*P* value
Atopy	N	78·2	85·9	0·105
	Y	87·2	102·6	
Asthma	N	79·6	87·3	0·319
	Y	82·5	100·2	
Atopic dermatitis	N	80·9	88·2	0·323
	Y	73·4	101·9	
Rhinoconjunctivitis	N	77·9	88·3	0·167
	Y	94·9	91·8	

Figure S1. Incidence of clinical cases per 100 person-trimesters in children under 15 years of age.

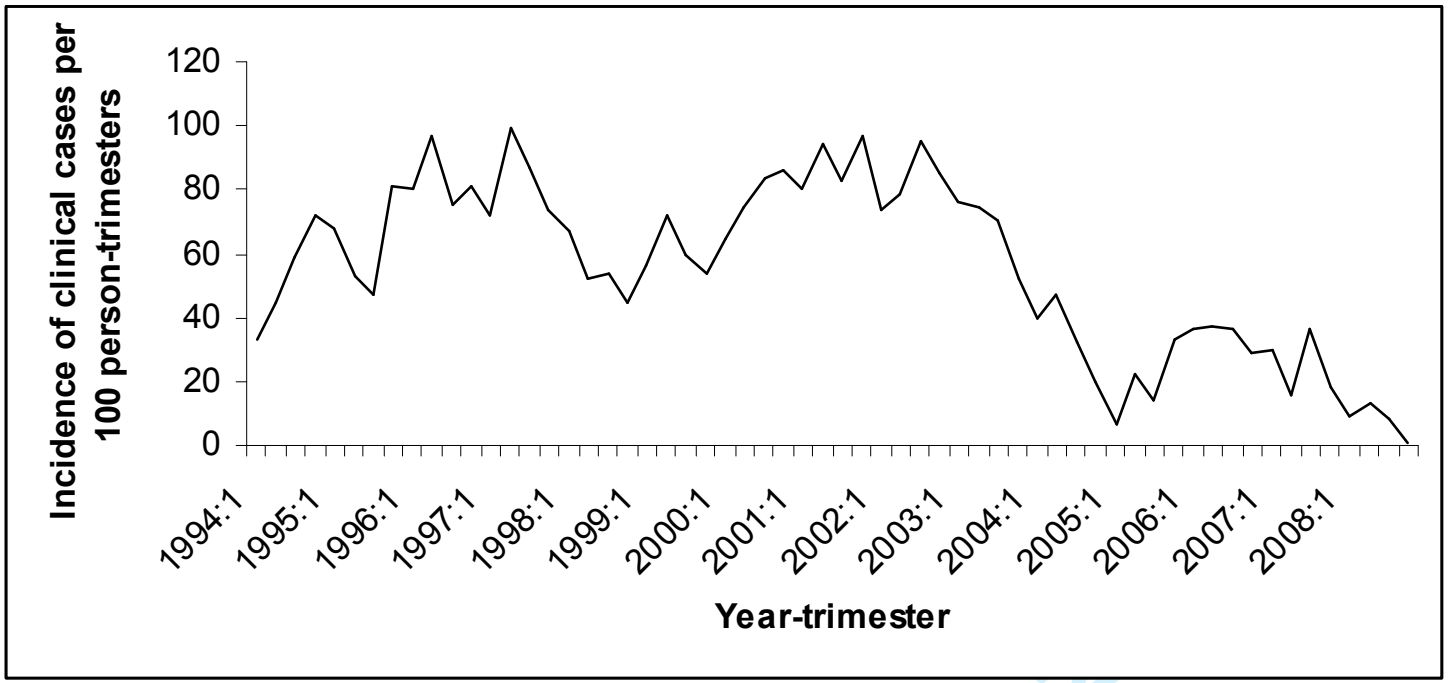


Figure S2. Cumulative incidence of clinical cases according to allergy class predicted by the statistical model.

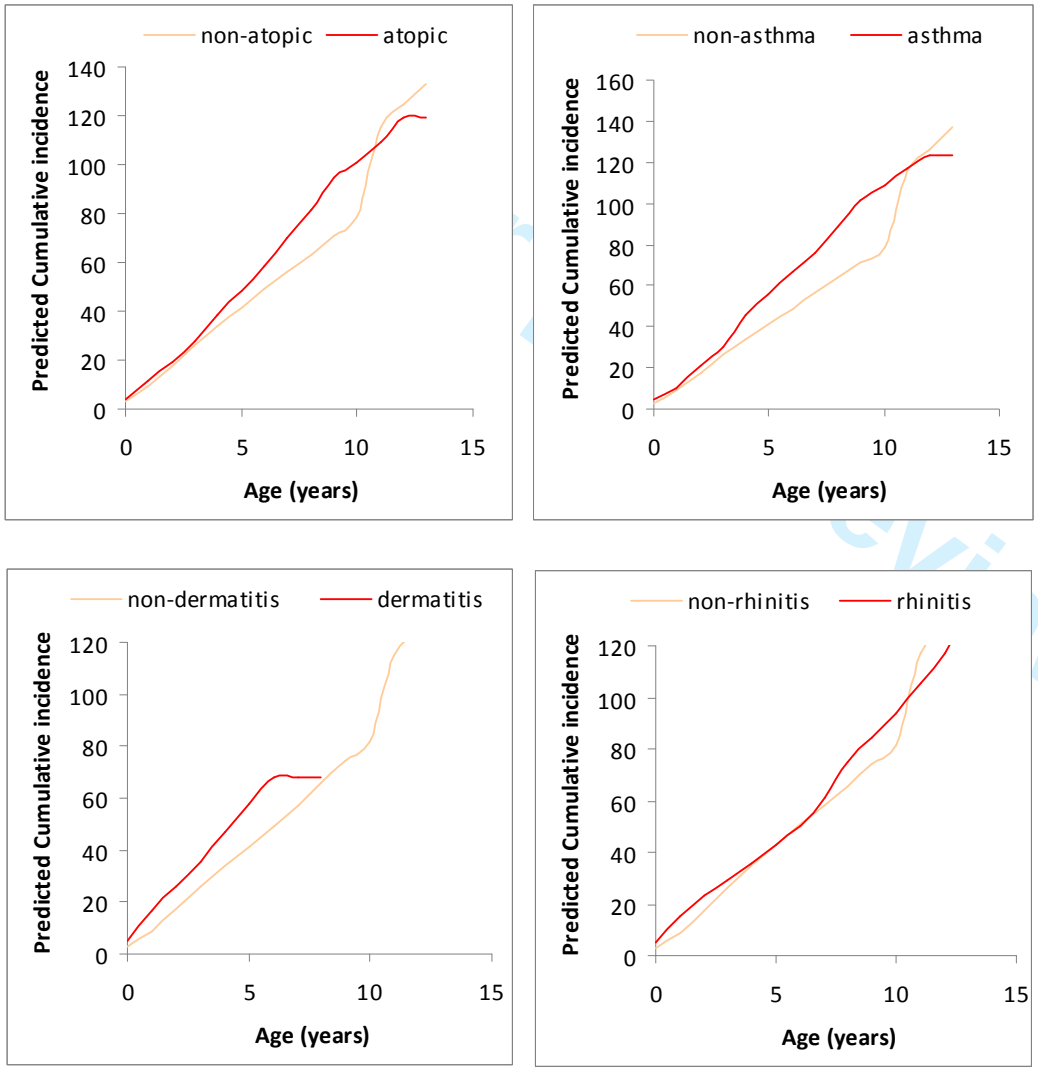
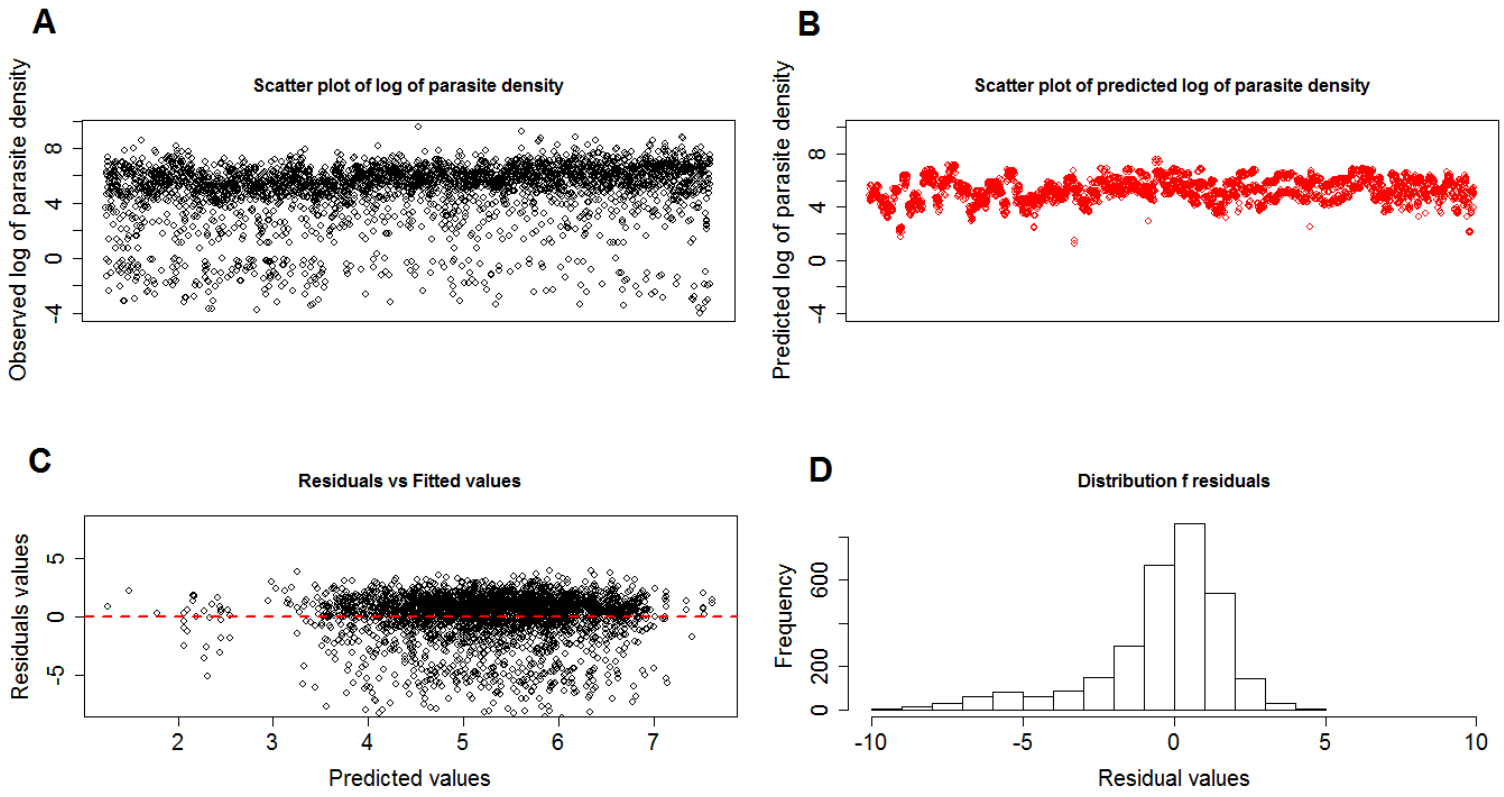


Figure S3. Graphical control model for parasite density

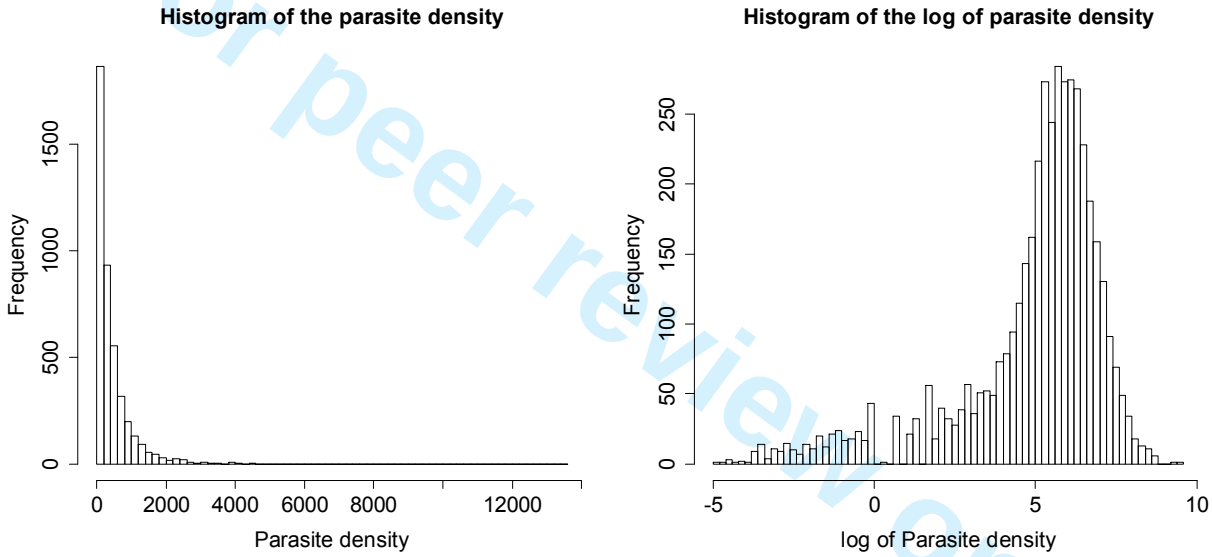
These figures provide a graphical checking of model goodness of fit. Figure A is the scatter plot of the natural logarithm of the observed parasite density and is compared to Figure B, which is the scatter plot of the natural logarithm of the predicted parasite density by the model; on both figures A and B the y-axes give the values for the log of the parasite density. Figure C shows the distribution of the residuals with the predicted values and Figure D is the histogram of the residuals; both figures C and D show the residuals normally distributed around zero.



Analysis using box-cox transformation and probit normalization

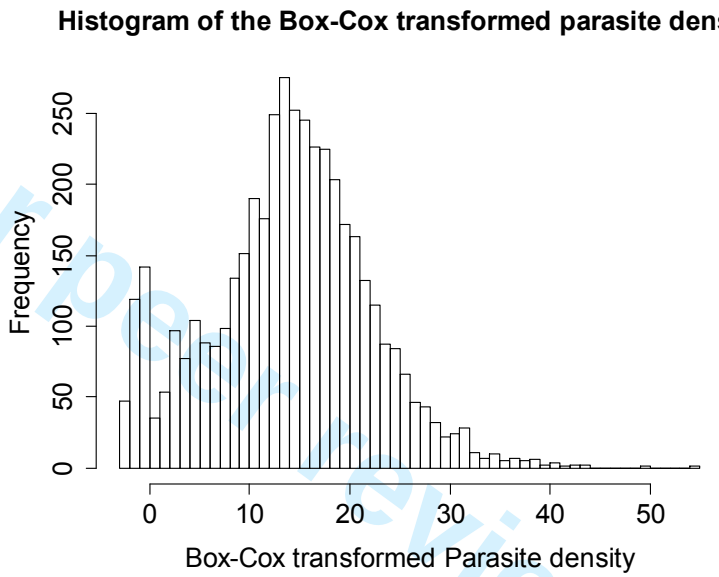
The model we fitted on the parasite density ("*pf_density*") has used as outcome variable the natural logarithm of *pf_density* (equivalent to a Box-Cox for which the parameter is null). As shown on Figure S4 the distribution of log(*pf_density*) is not perfectly normal, it is left-skewed.

Figure S4. Histogram of *pf_density* and log(*pf_density*)



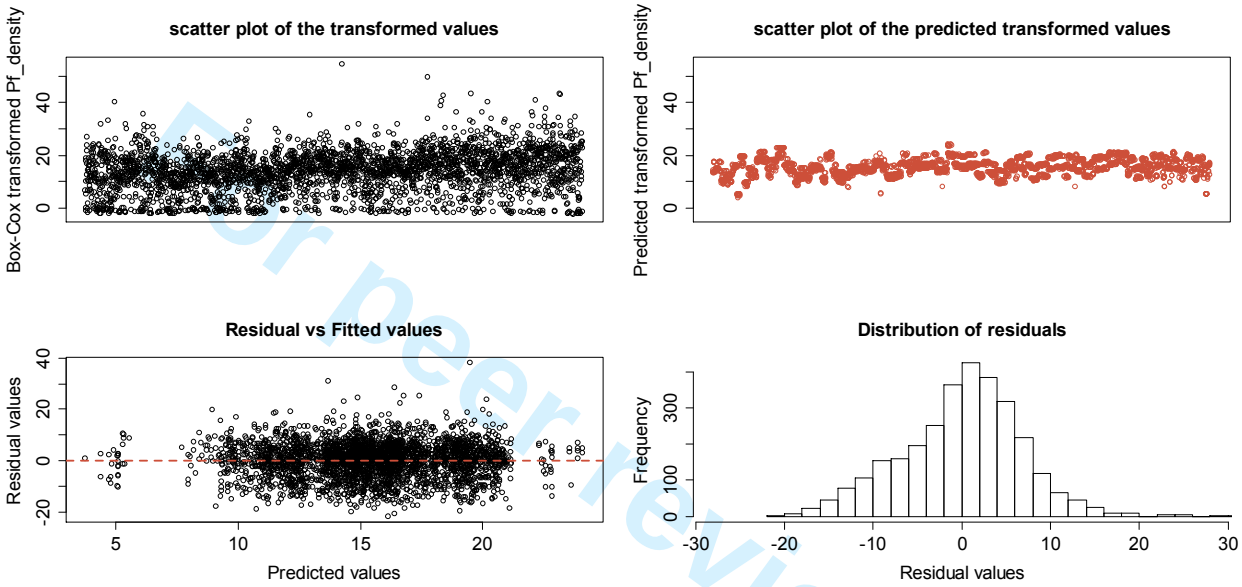
We add here the case for a Box-Cox transformation of the parasite density where the parameter is $\lambda = 0.3$, this parameter value was obtained as optimal using the R- function named "boxcox" from the "MASS" library. Then the Box-Cox transformation of the parasite density is $y = (pf\_density^{0.3} - 1)/0.3$ having the distribution shown on Figure S5 below.

Figure S5. Histogram of the Box-Cox transformation of pf_density using a λ parameter of 0.3



With this Box-Cox transformed parasitemia as outcome variable, our results are maintained. Note that this distribution is not "perfectly" normal. However, the corresponding graphical control of the model adequation presented on Figure S6 below shows residuals more close to the normal distribution than those for log(*pf_density*) as outcome.

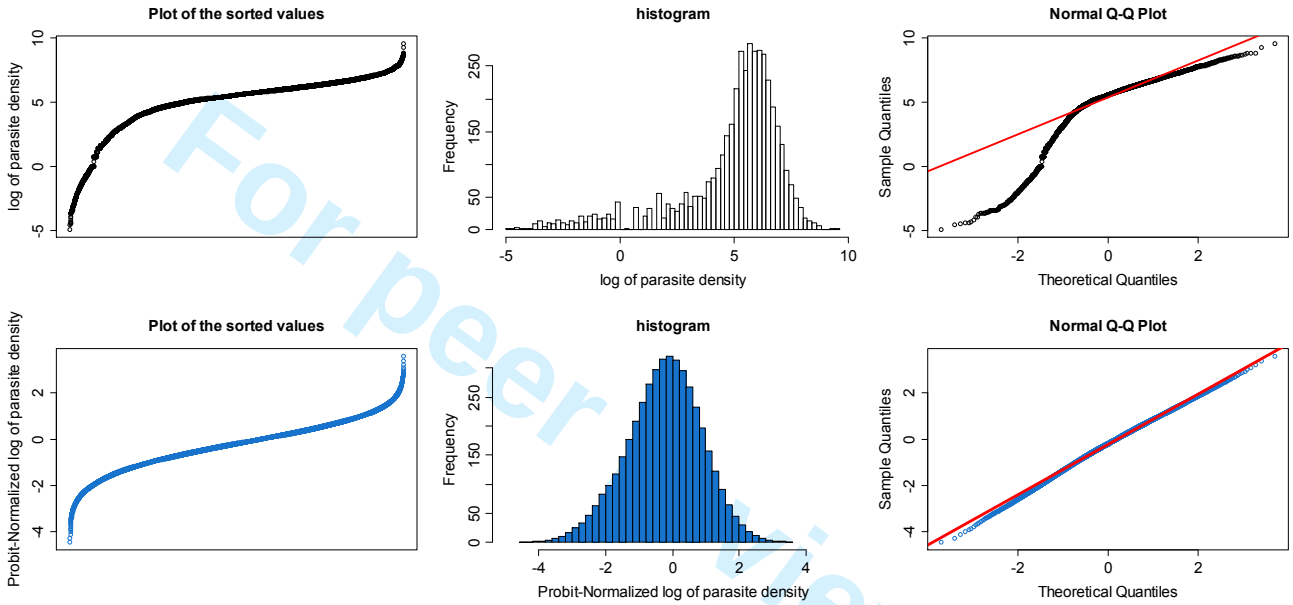
Figure S6. Graphical control of the model adequation for y = Box-Cox(*pf_density*, λ = 0.3)



Although using a mixed model approach based on an extreme value distribution would provide a more robust validation of these results, the method we used incorporating pedigree information was developed through an R-package known as "pedigreemm" that allows just for a limited number of distribution laws, which do not include extreme value distributions like the Gumbel or Weibull distributions.

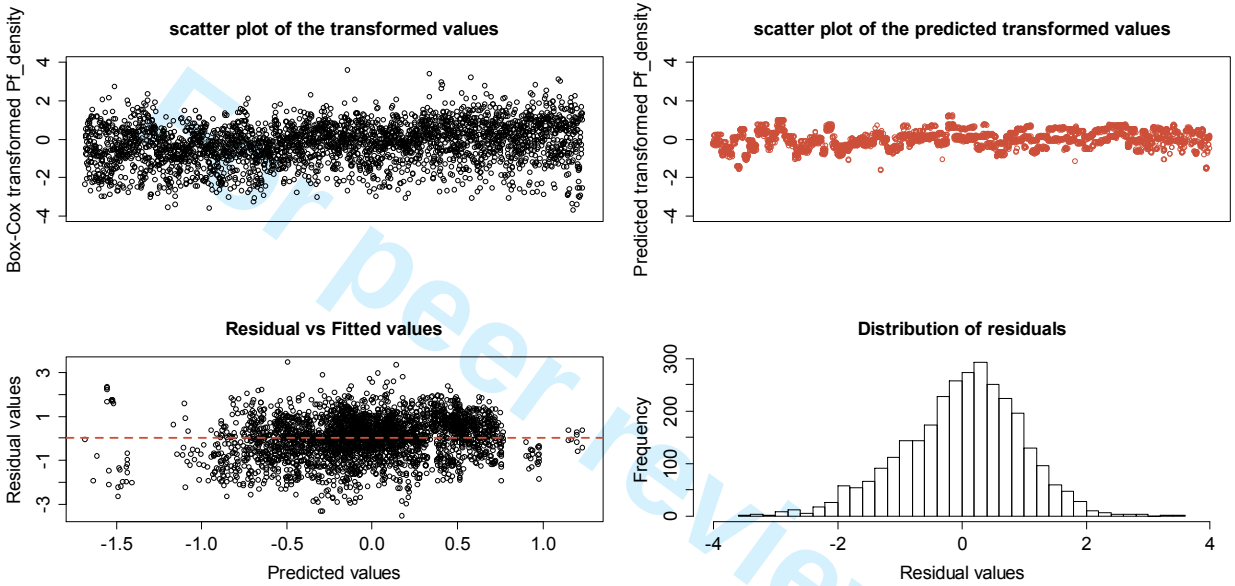
However, we tried the Probit normalization on the log(*pf_density*) to readjust its quantiles to those from a standard normal, and subsequently used the derived standard normal transformation of the log(*pf_density*) as outcome (see Figure S7 below, the three graphs presented in the first row of the graphs panel concern the log(*pf_density*) before Probit normalization and the three in the second row are for after Probit normalization. We can see on the histogram in blue color a good normal distribution of the y variable.

Figure S7. Probit normalization of the log(*pf_density*)



The results we obtained after this Probit normalization of the log(*pf_density*) confirmed the same findings. Also, the corresponding graphical control of the model adequation presented on Figure S8 below, shows a good normal distribution of residuals from this model.

Figure S8. Graphical control of the model adequation after Probit normalization of the log(*pf_density*)



Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium falciparum* malaria

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Article summary

Article focus

- Genetic studies suggest a link between susceptibility to allergy and malaria in Africa
- We hypothesize that atopy increases susceptibility to malaria

Key messages

- Results demonstrate an association between asthma, atopic dermatitis and susceptibility to clinical *P. falciparum* episodes.
- Genetic pre-disposition to asthma or atopic dermatitis impairs the acquisition of clinical immunity to malaria.
- Administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Strengths and limitations

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association.

Abstract

Objectives: To assess the impact of atopy and allergy on the risk of clinical malaria.

Design: A clinical and immunological allergy cross-sectional survey in a birth cohort of 175 children from 1 month to 14 years of age followed for up to 15 years in a longitudinal open cohort study of malaria in Senegal. Malaria incidence data were available for 143 of these children (aged 4 months to 14 years of age) for up to 15 years. Mixed model regression analysis was used to determine the impact of allergy status on malaria incidence, adjusting for age, gender, sickle cell trait and force of infection.

Main outcome measures: Asthma, allergic rhinoconjunctivitis and atopic dermatitis status, the number of clinical *Plasmodium falciparum* malaria episodes since birth and associated parasite density.

Results: Twelve percent of the children were classified as asthmatic and ten percent as having atopic dermatitis. These groups had respectively a two-fold (OR 2.12 95% confidence intervals 1.46 to 3.08; P= 8 $x10^{-5}$) and three-fold (OR 3.15, 1.56 to 6.33; P= 1.3 $x10^{-3}$) increase in the risk of clinical *P. falciparum* malaria once older than the age of peak incidence of clinical malaria (3 to 4 years of age). They also presented with higher *P. falciparum* parasite densities (Asthma: mean 105.3 parasites/µL ±SE 41.0 *vs*. 51.3 ±9.7; P= 6.2 $x10^{-3}$; Atopic dermatitis: 135.4 ±70.7 *vs*. 52.3 ±11.0; P=0.014). There was no effect of allergy on the number of non-malaria clinical presentations. Individuals with allergic rhinoconjunctivitis did not have an increased risk of clinical malaria nor any difference in parasite densities.

Conclusion: These results demonstrate that asthma and atopic dermatitis delay the development of clinical immunity to *P. falciparum*. Despite the encouraging decrease in malaria incidence rates in Africa, a significant concern is the extent to which the increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines, administration to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Introduction

The World Allergy Organization estimates that 40% of the world's population is concerned by allergic diseases.[1] In developing countries where *Plasmodium falciparum* malaria is endemic, prevalence of allergy is significantly lower, but is on the increase.[2] T helper type 2 (Th2) cells, their related cytokines, IgE, eosinophils and mast cells play a major role in allergic inflammation. Orientation of the immune response towards a Th1 profile is crucial for immunity to intracellular pathogens,[3] whereas orientation towards a Th2 profile drives immunity to extracellular pathogens and antigens resulting in class switching giving rise to IgE-producing B cells.[4] An important role of the Th1/Th2 balance in the development of clinical malaria following infection by *P. falciparum* has been suggested by numerous studies.[5-7] Whilst it is recognised that acquired anti-parasite immunity is IgG dependent,[8] It has been suggested that the Th2 bias induced by *P. falciparum* may exacerbate allergy parasite-specific IgE also impact upon the clinical outcome of infection.[8] For example, higher IgE but not IgG levels have been observed in patients with cerebral malaria than those with uncomplicated P. falciparum infection.[9] The role of IgE, however, remains unclear. [10] Likewise, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*.

THowever, the interplay between infectious agents and allergy is unclearambiguous. On the one hand, for example, severe respiratory syncytial virus infection in infants increased the risk of allergic rhinoconjunctivitis and allergic asthma.[11,12] On the other hand, measles,[13] hepatitis A[14] and tuberculosis[15] seemingly reduce atopy. Although, an atopic condition can increase incidence of disease, such as the case for the skin commensal *Staphylococcus aureus* in patients with atopic dermatitis,[16] an atopic tendency *per se* does not generally lead to increased illness from infectious agents.

Genome wide studies have identified chromosomal regions linked to clinical malaria, all of which overlap with those previously identified to be involved in atopic dermatitis, asthma, atopy and IgE levels,[17-19] suggesting that common mechanisms may be involved in both pathologies.[20] Chromosomal region 5q31 that has been repeatedly shown to be associated with control of parasite density and contains a cluster of cytokines, among which IL12B has been previously associated with psoriasis.[21] The other regions, 13q13-q22, 5p15-p13 and 12q21-q23, contain genes involved in innate immunity, notably the interleukin 7 receptor,

and several involved in tumour necrosis factor synthesis [C1q and tumour necrosis factor related protein 3 (C1QTNF3)] and a gene involved in the complement system (C9). [20,18]

Several additional lines of evidence support the concept that susceptibility to malaria and atopy may be related to similar immunological defects. In Ethiopia, a history of malaria was associated with atopy.[22,0] A mouse model for human atopic disease was found to be very susceptible to murine malaria and a major locus for atopic disease mapped close to the region controlling parasite density.[23,1] This region contains several candidate genes that have effects on T-cell function.[23,1]

Moreover, a direct effect of histamine in the malaria pathogenesis has been found using genetic and pharmacological approaches[24,2] and increased levels of histamine are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[25,3,26,4]

To test the hypothesis that allergy impacts upon clinical *P. falciparum* malaria, we performed a clinical allergy cross-sectional study in the family-based longitudinal cohort from Senegal previously used for the genome linkage study[20,18] and analysed the impact of asthma, atopic dermatitis, allergic rhinoconjunctivitis on the incidence of clinical *P. falciparum* episodes and the maximum parasite density during each episode.

Methods

Population and outcome data

The malaria research program conducted in Dielmo village in Senegal has been ongoing since 1990 as described elsewhere.[27,5] In brief, between 1990 and 2008, a longitudinal study involving the inhabitants of the village of Dielmo, Senegal, was carried out to identify all episodes of fever. The study design included daily medical surveillance with systematic blood testing of individuals with fever and examination of 200 oil-immersion fields on a thick blood film for malaria parasites (about 0.5 µL of blood). Each individual was given a unique identification code and details of family ties, occupation, and precise place of residence were recorded on detailed maps of each household with the location of each bedroom. All households were visited daily, absenteeism recorded, and the presence of fever or other

symptoms assessed. We systematically recorded body temperature at home three times a week (every second day) in children younger than 5 years, and in older children and adults in cases of suspected fever or fever-related symptoms. In cases of fever or other symptoms, blood testing was done at the dispensary by finger prick, and we provided detailed medical examination and specific treatment. Parasitologically confirmed clinical malaria episodes were treated according to national guidelines. From 1990 to 2008, four different drug regimens were implemented: Quinine from 1990 to 1994, Chloroquine from 1995 to 2003, Fansidar (sulfadoxine-pyrimethamine) from 2004 to mid-2006 and Artemisinin-based combination therapy (ACT; Amodiaquine- sulfadoxine-pyrimethamine) from mid-2006 to 2008.

Parasite positivity was established as follows. Thick blood films were prepared and stained by 3% Giemsa stain. Blood films were examined under an oil immersion objective at x1000 magnification by the trained laboratory technicians and 200 thick film fields were examined to count the number of asexual and gametocyte parasite stages. Asexual parasite densities (per µL) were calculated by establishing the ratio of parasites to white blood cells and then multiplying the parasite count by 8,000, the average white blood cell count per µL of blood.

Malaria transmission in Dielmo is intense and perennial. We conducted a cross-sectional survey to estimate the prevalence of symptoms related to allergic diseases among 175 children aged from 1 month to 14 years old who were born during the malaria research program.

Both the longitudinal and cross-sectional surveys were approved by the Ministry of Health of Senegal. Informed consent of the volunteers is renewed every year. More specifically for the cross-sectional survey, after informing about the procedures and the purpose of the study, written informed consent was obtained from parents or guardians of children either by signature or by thumbprint on a voluntary consent form written in both French and Wolof, the main local language. Consent was obtained in the presence of the school director, an independent witness.

The family structure (pedigree) was available after a demographic census performed for every volunteer at his adhesion in the project. A verbal interview of mothers or key representatives of the household was used to obtain information on genetic relationships between studied individuals, their children, their parents, and to identify genetic links

among the population. The total pedigree comprised 828 individuals, including absent or dead relatives, composed of ten independent families that can be sub-divided into 206 nuclear families (father – mother couples with at least one child) with an average of 3.6 children each. Genetically related nuclear families occur because of multiple marriages and marriages among related individuals. Previous typing with microsatellites has enabled the construction of a pedigree based on Identity-by-Descent using MERLIN.[2018,286] The mean coefficient of inbreeding is 0.0008. Newborns since this original genetic analysis were added to the family of the parents in question. The 143 children, with both allergy and malaria data, belonged to 61 nuclear families and comprised 30 singletons, 102 siblings and 11 half-sibs (yielding 55 half-sib pairs). The mean genetic relatedness (by pedigree) of the 143 children is 0.0114 (range: 0.0013 to 0.022).

P. falciparum *clinical episodes*

P. falciparum malaria clinical episode phenotypes analysed were: (i) clinical *P. falciparum* infections treated with anti-malarial therapy and (ii) the highest parasite density during the *P. falciparum* clinical episode. A clinical *P. falciparum* episode was defined as a clinical presentation with fever (axillary temperature ≥ 37.5°C) and/or other clinical signs suggestive of malaria associated with a thick blood smear positive for *P. falciparum* and that was treated with anti-malarial therapy. Repeated clinical malaria presentations within 15 consecutive days were not considered to be independent and were excluded from the analyses, unless there was a negative thick blood smear between two clinical presentations. We also excluded observations in any trimester for which the individual was not present for at least one third of the time.

We calculated the quarterly incidence rate of clinical *P. falciparum* episodes in children below the age of 15 years as the ratio of the total number of clinical *P. falciparum* episodes during the trimester divided by the total number of person-trimesters surveyed. Incidence rate is expressed as cases per 100 person-trimesters (see Supplementary Figure S1). This rate was used in the analysis to approximate the force of infection (exposure level) within the targeted population at the time of a given clinical *P. falciparum* episode.

The total number of clinical presentations per trimester that were not attributable to *P. falciparum* was tabulated. Repeated non-malaria presentations within seven consecutive days were not considered to be independent and were excluded.

Allergic diseases and atopic status

The International Study of Asthma and Allergies in Childhood (ISAAC) diagnostic criteria have been shown to be reproducible, adequate and able to discriminate children with allergic diseases in different areas of the world.[2] The standardized ISAAC questionnaire originally written in English was translated into French in compliance with ISAAC guidelines[2~~97~~], adapting it to the usual local customs following advice from local clinicians and paediatric allergologists (Acknowledgements and Technical Appendix). The adequacy and reliability of the translated questionnaire had been previously confirmed by a pilot study on 30 randomly selected children in the same community. The questionnaire was completed by specially trained health workers during an oral interview conducted in Wolof with children and their mothers or guardians.

To assess the prevalence of allergic diseases in children, we used the positive and negative predictive values of the ISAAC questionnaire diagnosis criteria developed for subtropical countries.[30~~28~~] Each question was scored according to the medical diagnosis of paediatricians and paediatric allergologists. Positive or negative answers were thus graded on the basis of symptom sensitivity, specificity, frequency, location or early onset. For each allergic disease, three categories of symptom severity, *severe*, *moderate*, and *none*, were defined as follows:

Asthma – severe symptoms if the child had "wheezing or whistling in the chest before the age of two years" and "more than three times" *or* severe enough to "limit his/her speech"; *moderate* symptoms if the child had "wheezing or whistling in the chest before the age of two years" and "in the past 12 months"; and *none* otherwise.

Allergic rhinoconjunctivitis – severe symptoms if the child had "sneezing, runny or stuffy nose in the past 12 months" and "more than five times a year", and "itchy, watery eyes or tropical endemic limboconjunctivitis (TELC) in the past 12 months"; *moderate* symptoms if the child had "sneezing, runny or stuffy nose in the past 12 months", and "itchy, watery eyes or TELC in the past 12 months"; and *none* otherwise.

Atopic dermatitis – severe symptoms if the child had "scaly or exudating, crusted and pruritic patches in the past 12 months" and "affecting any of the following characteristic areas: face, around the ears or eyes, folds of armpits or elbows or groin, behind the knees, under the buttocks", and "onset of symptoms before the age of two years"; *moderate* symptoms if the

child had “scaly or exudating, crusted and pruritic patches in the past 12 months” and “affecting any of characteristic areas (see above)”, and “onset of symptoms before the age of four years”; and *none* otherwise.

The inter-relationships between variables reflecting the severity of symptoms of the three allergic diseases were used to identify children at high risk of atopy. The *high probability* group was defined by the prevalence of at least one of any *severe* symptoms or two of any *moderate* symptoms. The *probable* group was defined as those with *moderate* symptoms from one of the three allergic diseases and remaining children were classified in the *unlikely* group.

Helminths

Helminthic infections are common in this region and are known to modify the clinical course and outcome of both allergic diseases and malaria.[31,32] We therefore carried out a helminth survey for 91 individuals present during the cross-sectional survey. Diagnosis was performed by stool examination by microscope and by the Kato technique to search for the presence of *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*), whipworm (*Trichuris trichiuria*), *Schistosoma mansoni*, and *Strongyloides stercoralis*. Examination for pinworms (*Enterobius vermicularis*) was performed by the anal scotch-test. An anti-helminthic treatment was proposed for all infested individuals.

Immunoglobulin E titres

Specific IgE titres were measured by ELISA as previously described.[33] A panel of allergens of potential pertinence to the three classes of allergy was used: (i) Salivary gland extracts (SGE) of two mosquito species present in the study cohorts, *Aedes aegypti* and *Anopheles gambiae sensu stricto*, and (ii) *P. falciparum* parasite extract were prepared as previously described[31]; (iii) House dust mite spp. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*; (iv) a mix of pollen allergens from five ubiquitous graminae spp. [Cock’s-foot (*Dactylis glomerata*), Timothy grass (*Phleum pratense*), Sweet Vernal grass (*Anthoxanthum odoratum*), Perennial ryegrass (*Lolium perenne*), Kentucky Bluegrass (*Poa pratensis*)] (all from Stallergenes, France).

Statistical analysis

Statistical analyses were performed using R version 2.12.0 (The R Foundation for Statistical Computing, Vienna, Austria). To address the effect of allergic status on the risk of clinical *P. falciparum* episodes, we performed Generalized Linear Mixed Models (GLMM) extended to pedigree data using the *pedigreemm* package for R to account for the non-independence of individuals because of family relationships, shared house and for repeated measures from the same individual (Technical Appendix). Correlated individual effects due to familial relationships were taken into account by using the pedigree-based genetic relatedness matrix that contains the genetic covariance among all pairs of individuals in the study cohort and is calculated using the pedigree information.[34~~2~~] Shared house and repeated measures from the same individual were modelled as random effects. All random effects were assumed to be normally distributed, and conditional on these random effects, the dependent variable had: (i) a Binomial distribution when the studied phenotype was the occurrence of a clinical *P. falciparum* episode treated with anti-malarial therapy during a trimester, (ii) a Gaussian distribution when the studied phenotype was the logarithm of the maximum parasite density during a given clinical *P. falciparum* episode, and (iii) a Poisson distribution when the studied phenotype was the number of non-malaria episodes per trimester. The effects of allergy disease classes on these dependent variables were modelled as fixed effects. Allergy classes were reduced to two levels, *Severe* or *moderate vs*. *none* for analyses of asthma, atopic dermatitis and allergic rhinoconjunctivitis and *high probability vs*. *probable* and *unlikely* for atopic tendency. Co-variables included sickle cell trait[33~~1~~], gender, number of days present on site during the trimester, trimestrial incidence of *P. falciparum* and age. Age was initially analysed as a continuous covariate. To assess the age-specific effect of allergy, age was categorised into two levels (<3.5 years of age and ≥3.5 years of age, based on the age of peak clinical incidence) and allergy class was nested within age class. The age threshold was varied from 1.5 years to 5.5 years of age and the data re-analysed to assess at which age there was the strongest effect. The association of allergy classes with IgE levels was analysed by box-cox transforming the data and fitting a GLMM with a normal distribution.

Results

Of the 205 eligible children aged under 15 years involved in the family-based longitudinal study, 175 (85.4 %) participated in the cross-sectional survey to assess the prevalence of related symptoms of allergic diseases. All eligible children present at the time of the survey were included; no explicit refusal to participate was recorded. The study cohort was aged from 1 month to 14 years 11 months. The sex-ratio (male/female) was 0.94.

From 1994 until 2008, 143 of the children participating in the cross-sectional survey were present for at least 31 days in any trimester during the study period generating a total of 3,093 person-trimesters of presence (Supplementary Table S1). There were 2,065 treated *P. falciparum* clinical episodes (per individual: median 11, range 0-47)(Supplementary Table S2). The age peak of incidence of *P. falciparum* episodes occurred at 3 to 4 years of age (Figure 1). There were 1,868 non-malaria episodes (median 12, range 0-37) (Table S2). These non-malaria clinical presentations were associated with headache (38 %), chills (32 %), cough (13 %), vomiting (11 %) and diarrhoea (6 %).

The prevalence of moderate or severe asthma symptoms was respectively 2.3 % and 10.3 % (Table 1). The prevalence of moderate or severe allergic rhinoconjunctivitis symptoms was respectively 6.3 % and 10.3 %. The prevalence of moderate or severe atopic dermatitis symptoms was respectively 6.3 % and 2.9 %. On the basis of symptom severity, an atopic tendency was estimated to be unlikely for 68.0 %, probable for 9.1 % and highly probable for 22.9 % of the 175 children. The frequency of each allergy class in children for whom malaria data were available is shown in Table S1.

The risk of treated clinical *P. falciparum* infections was higher for children with high probability of atopy (OR 1.65, 95% confidence intervals 1.20 to 2.26; P=0.002) (Table 2), after adjusting for age, sickle cell trait and the exposure level. Gender was not found to be significant. Analysing the impact of atopy in children younger and older than the peak age of clinical incidence (3 to 4 years old), revealed that atopy increased the risk of *P. falciparum* episodes in children at an age greater than 3.5 years (OR 2.02, 1.39 to 2.93; $P=2x10^{-4}$), but not in children of age prior to the peak clinical incidence (OR 1.38, 0.92 to 2.08; P=0.124) (Table 2). This increased risk resulted in an ever increasing cumulative number of *P. falciparum* episodes with age beyond that of peak clinical incidence (Figure 2. See supplementary Figure S2 for model predictions for comparison).

Analysis by allergy category revealed that asthma (severe or moderate) increases the risk of *P. falciparum* episodes (OR 2.12, 1.46 to 3.08; P= 8 x10^{-5}) and this again only in children of age greater than 3.5 years old (OR 2.33, 1.50 to 3.61; P= 1.5 x10^{-4}). Atopic dermatitis increased the risk of clinical malaria in children older (OR 3.15, 1.56 to 6.33; P= 1.3 x10^{-3}) but not younger than 3.5 years of age (Table 2). Allergic rhinoconjunctivitis was not associated with increased risk of clinical malaria at any age (Table 2). The impact of atopy, asthma and atopic dermatitis can be clearly seen in the ever-increasing number of cumulative *P. falciparum* episodes beyond the age of the onset of clinical immunity in the population, 3.5 years of age (Figure 2). There is no difference in the number of clinical malaria episodes prior to this age in individuals with or without an allergic condition. Analysis using different age thresholds (from 1.5 to 5.5 years of age) revealed similar OR for thresholds of 2.5, 3.5 and 4.5 years of age. The maximum OR for increased malaria occurred in children older than 4.5 years of age and with atopy or atopic dermatitis, whereas for the asthma group it occurred in children after 3.5 years of age (Supplementary Table S3).

There was no impact of any allergic disease on the number of non-malaria episodes by trimester (Supplementary Table S4).

The impact of atopy, asthma and atopic dermatitis on the maximum *P. falciparum* parasite density during a given clinical malaria episode mirrored that of the risk of *P. falciparum* episodes. Parasite density was significantly higher for children with allergic disease older than 3.5 years of age (Table 3 and supplementary Figure S3 for residuals of the fitted model). As the log-transformed data were left skewed, we additionally analysed using box-cox transformation and probit normalization of the data. The results were qualitatively the same (Supplementary text and Figures S4-S8). Allergic rhinoconjunctivitis had no impact on the parasite density (Table 3). Analysis using different age thresholds yielded ~~the same pattern~~similar qualitative conclusions as seen with the number of clinical episodes (Table S3).

Individuals with moderate or severe symptoms of atopic dermatitis had significantly higher specific IgE titres against *Ae. aegypti* (P=0.004) and *An. gambiae* SGE (P<0.001). There were no detectable specific anti-*P. falciparum* IgE. Individuals with moderate or severe symptoms of allergic rhinoconjunctivitis did not have significantly higher IgE titres against the tested graminae (P=0.28), although titres decreased with age (P=0.035). There was also no effect of

asthma on IgE titres against the house dust mite spp. tested (*D. farinae* P=0.60 & *D. pteronyssinus* P=0.27).

Only five individuals were infested with helminths (two *Ancylostoma*, one *Strongyloides*, one *Trichuris* and one *Enterobius*).

Discussion

Principal findings

Establishing the allergic status of children up to the age of 15 years old followed for malaria since birth, revealed an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

Strengths and weaknesses of the study

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association. In addition, although allergy diagnosis for children under 2 years of age is not considered reliable, there were only 15 individuals under 2 at the time of the allergy study of the 143 for whom malaria and allergy data were available.

Meaning of the study

Under intense malaria transmission, after repeated exposure to the parasite, children develop a clinical immunity[3,5], whereby they tolerate elevated parasite densities without showing clinical symptoms. In this cohort, the population mean onset of clinical immunity occurred at 3 to 4 years of age. Although clinical immunity is accompanied by a reduction in parasite density, effective anti-parasite immunity develops much more slowly[3,6,4] with individuals achieving a state of premunition, whereby they maintain low-grade parasite densities in an asymptomatic state.[3,7,5] We show here that children with clinically defined

asthma or atopic dermatitis ~~had a two to three-fold~~have an increased ~~in the~~ risk of presenting with *P. falciparum* malaria episodes requiring treatment once passing the age of peak clinical incidence. They also had higher parasite density during clinical episodes, suggesting a reduced ability to control parasite replication. The observed increase in clinical incidence of malaria in patients with asthma or atopic dermatitis is not likely to be the result of increased frailty of such individuals; these individuals did not come more frequently to the clinic with non-malaria symptoms. Our previous genome linkage study identifying chromosomal regions[20~~18~~] associated with malaria that overlap with those previously shown to be linked to asthma/atopy suggests that there may be a shared genetic basis to these pathologies rather than any causative effect of one on the other. This is consistent with the increased susceptibility to malaria of mouse atopic models.[2~~31~~]

Comparison with other studies

A previous study in Ethiopia (East Africa) found that a history of malaria (yes/no) increased risk of atopic dermatitis in 306 cases compared to 426 controls as characterized using the ISAAC questionnaire.[2~~20~~] The only other epidemiological study that has previously examined the link between malaria and atopy[3~~86~~] also interpreted the result from the perspective of the impact of malaria on atopy. They examined the re-infection rate with *P. falciparum* over a 5-year period in 91 children that were subsequently classified as atopic or not using skin prick tests (SPT) with house dust mite antigen. Their conclusion was that, as with measles[13~~1~~] and tuberculosis[15~~3~~], malaria infection reduces atopy. However, the study lacked previous infection data since birth of the participating individuals and focussed on atopy as determined by SPT against a single allergen. The case-control study of atopic dermatitis risk factors cited above found no overall association between allergen skin sensitization and atopic dermatitis. We also found no evidence of increased IgE titres against house dust mites in the asthmatic or atopic dermatitis groups or against grass pollen in individuals with allergic rhinoconjunctivitis. Such differences likely reflect the different IgE reactivity profiles due to differences in allergen exposure in Africa.[39~~7~~] There was no evidence of anti-parasite IgE in this cohort of children. We previously showed that circulating anti-parasite IgE titres were strongly positively correlated with anti-mosquito saliva IgE, but became undetectable following malaria exposure, potentially being bound to effector cells.[33~~1~~] Only mosquito

saliva, a known major local allergen, induced a specific IgE response at significantly higher titres in individuals with atopic dermatitis.

Although the immune effectors of clinical immunity are still poorly defined, there is strong evidence that acquired anti-parasite immunity is IgG-dependent[38] and cytophilic immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by opsonisation and/or by Antibody Dependent Cellular Immunity play an important role in premunition.[375] The higher parasite density during symptomatic episodes observed in the asthma group suggests impaired development of acquired immunity. Impaired acquisition of immunity to malaria in children with asthma or atopic dermatitis may stem from their imbalanced Th1/Th2 response. Indeed, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*. Dendritic cells that are oriented to a Th2 phenotype are more susceptible to orient the acquired immune response towards a Th2 profile.[4039] Orientation of the immune response towards a Th2 profile by asthma or atopic dermatitis would result in a poor Th1 response (and hence development of protective IgG immunoglobulins), considered to be the dominant arm of the immune response enabling resistance to infectious disease in children.[410]

Many studies have revealed an important role of histamine, a key downstream effector molecule in allergic reaction, in the outcome of a malaria parasite infection.[242-264,421-454] Moreover, reports indicate that components of the innate immune system, including eosinophils, basophils, and mast cells (MCs), could play important roles in the pathogenesis of malaria.[421] Increased levels of histamine in plasma and tissue, derived from basophils and MCs, notably following stimulation by IgE through the high affinity receptor FcεR1, are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[253,264] Chlorpheniramine, a HR1agonist reversed resistance to chloroquine and amodiaquine both *in vivo* and *in vitro*.[432] Moreover, astemizole, another HR1 agonist, was identified as an anti-malarial agent in a clinical drug library screen.[443] Finally, *P. falciparum* produces translationally controlled tumor protein, which is a homolog of the mammalian histamine-releasing factor that causes histamine release from human basophils.[454]

Further research

Our results provide the first birth cohort study addressing the link between malaria and allergic diseases. They contribute to a growing body of evidence that the pathologies are related. ISAAC has revealed a steady but significant increase in prevalence rates of asthma and allergic diseases in Africa. Whilst the majority of studies have focused on large cities, there is increasing urbanization throughout Africa, as well as improved access to primary health care in many areas. A key concern for ISAAC is the extent to which such societal evolution will result in an increase in allergic diseases. Increased urbanization in sub-Saharan Africa is changing the epidemiology of malaria and although resulting in a decrease in risk, will result in more severe clinical malaria in older individuals.[4,5,4,6] Moreover, a large consumption of anti-malarial drugs in the urban areas provides substantial drug pressure fostering, the selection of drug-resistant parasites. Despite the encouraging recent decrease in malaria incidence rates, even in rural areas, an additional significant concern is the extent to which such an increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines[47,48], administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials[49,8] and alleviate the non-infectious consequences of atopy. Clinical intervention studies should be envisaged.

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What is already known on this topic

There are several reports of the beneficial effects of anti-histamines for malaria chemoprophylaxis[2,42-2,64,48,7] as well as our previous work[20,18] showing that chromosomal regions associated with malaria are also linked to allergy and atopy.[1,75-1,97] There are two epidemiological studies showing opposite effects of malaria on atopy.[2,20,3,86]

What this study adds

Using a longitudinal malaria study birth cohort, we identified an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred only after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

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Contributors: LB, SM, and RP made substantial contributions to the concept and design of the study. MH, HB, BG, SB, FDS, and AT were involved in acquisition of the data. CL, AF, OMP, AS and RP contributed to the analysis and interpretation of the data. MH, CL, HB, BG, SB, FDS, AF, AT, LB, OMP, SM, AS and RP critically reviewed the report and approved its final version for submission. All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. MH and RP are guarantors.

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: The allergy study was approved by the Senegalese National Ethics committee (2009/N°46). Renewed approval of the longitudinal malaria study was obtained from the same committee (2006/N°969).

Data sharing: The allergy database will be made available on-line. The longitudinal malaria data set will be made available following discussion with the coordinators of the three Institutes that govern the dataset through contact with the corresponding author.

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Table 1 Classification of Asthma, Allergic rhinoconjunctivitis, Atopic dermatitis and overall Atopic status according to ISAAC questionnaire in children aged 0-14 from a malaria birth cohort. N is total number of children examined and n-malaria represents those for whom malaria data were recorded. F is the number of females and M the number of males.

	N (F/M)	%	n-malaria (F/M)
Asthma symptoms			
None	153 (73/80)	87.43	125 (59/66)
Moderate	4 (1/3)	2.29	4 (1/3)
Severe	18 (6/12)	10.29	14 4/10)
Rhinoconjunctivitis symptoms			
None	146 (64/82)	83.43	120 (52/68)
Moderate	11 (8/3)	6.29	9(6/3)
Severe	18 (6/12)	10.29	14 (6/8)
Atopic dermatitis symptoms			
None	159 (75/84)	90.86	128 (60/68)
Moderate	11 (1/10)	6.29	11 (1/10)
Severe	5 (4/1)	2.86	4 (3/1)
Atopic tendency			
Unlikely	119 (56/63)	68.00	97 (46/51)
Probable	16 (8/8)	9.14	14 (6/8)
Highly probable	40 (16/24)	22.86	32 (12/20)

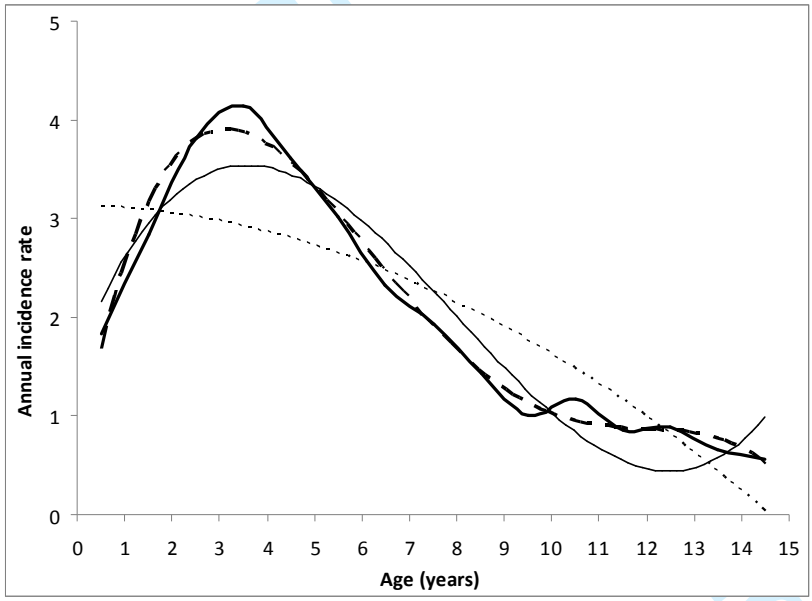
Table 2 Impact of allergy status on risk of *P. falciparum* clinical episodes. Shown are the *P* values and adjusted Odds Ratios with 95% confidence intervals calculated from the mixed model analyses. Values for the covariables Age (≥3.5 years of age compared to <3.5 years of age), Trimestrial incidence of *P. falciparum* clinical episodes and HbAS (beta-globin sickle cell trait; AS compared to AA) are those from the Asthma model analysis. For clarity significant co-variables are shown in bold.

	Age groups <3.5 years>	ORa	95% Confidence Intervals		*P* value
			Lower	Upper	
Atopy	**Both**	**1.65**	**1.20**	**2.26**	**2.0×10^{-3}**
	<3.5	1.38	0.92	2.08	0.124
	≥3.5	**2.02**	**1.39**	**2.93**	**2.1×10^{-4}**
Asthma	**Both**	**2.12**	**1.46**	**3.08**	**8.0×10^{-5}**
	<3.5	1.50	0.90	2.50	0.122
	≥3.5	**2.33**	**1.50**	**3.61**	**1.5×10^{-4}**
Atopic dermatitis	Both	1.05	0.65	1.70	0.842
	<3.5	0.84	0.49	1.46	0.539
	≥3.5	**3.15**	**1.56**	**6.33**	**1.3×10^{-3}**
Rhinoconjunctivitis	Both	0.96	0.65	1.41	0.818
	<3.5	1.05	0.64	1.72	0.853
	≥3.5	0.95	0.60	1.52	0.834
Age ≥3.5		**0.48**	**0.40**	**0.57**	**2.7×10^{-15}**
Trimestrial incidence		**1.01**	**1.00**	**1.01**	**1.8×10^{-6}**
HbAS		**0.24**	**0.12**	**0.47**	**3.7×10^{-5}**

Table 3 Impact of allergy status on the maximum *P. falciparum* parasite density during a clinical malaria episode. Shown are the back-transformed mean parasite densities per microlitre and standard errors (SEM) estimated from the GLMM analyses after taking into account the other co-variables. Significantly different effects are shown in bold for clarity.

Allergic condition	Age groups	Allergic status (No/Yes)	Mean parasite density	SEM	*P* value
Atopy	**Both**	**N**	**76.3**	13.8	
		Y	**131.0**	36.4	**0.0158**
	<3.5	N	114.3	23.7	
		Y	171.1	56.0	0.148
	≥3.5	**N**	**48.4**	9.8	
		Y	**114.8**	37.1	**$9.5x10^{-4}$**
Asthma	**Both**	**N**	**78.1**	14.4	
		Y	**148.5**	44.3	**$3.8\ x10^{-3}$**
	<3.5	N	114.8	24.3	
		Y	171.9	74.5	0.167
	≥3.5	**N**	**51.3**	9.7	
		Y	**105.3**	41.0	**$6.2\ x10^{-3}$**
Atopic dermatitis	Both	N	82.6	15.0	
		Y	93.9	38.9	0.605
	<3.5	N	122.6	25.5	
		Y	133.9	63.5	0.425
	≥3.5	**N**	**52.3**	11.0	
		Y	**135.4**	70.7	**0.014**
Rhinoconjunctivitis	Both	N	81.5	14.8	
		Y	111.4	39.0	0.570
	<3.5	N	118.8	25.1	
		Y	166.3	69.9	0.537
	≥3.5	N	54.6	11.3	
		Y	80.9	33.7	0.327

Figure 1 Annual incidence rate of clinical *P. falciparum* episodes per 100 children (bold line). In order to overcome the fluctuations of the annual incidence rate, we fit second (dotted line), third (dashed line) and fourth (solid line) degree polynomial trend lines to the data (bold line). The corresponding R-squared values are 0.70, 0.91 and 0·99 respectively indicating an accurate fit for third and fourth order polynomials. The inflexion on these two trend lines indicates the onset of acquisition of clinical immunity at approximately 3 to 4 years of age.



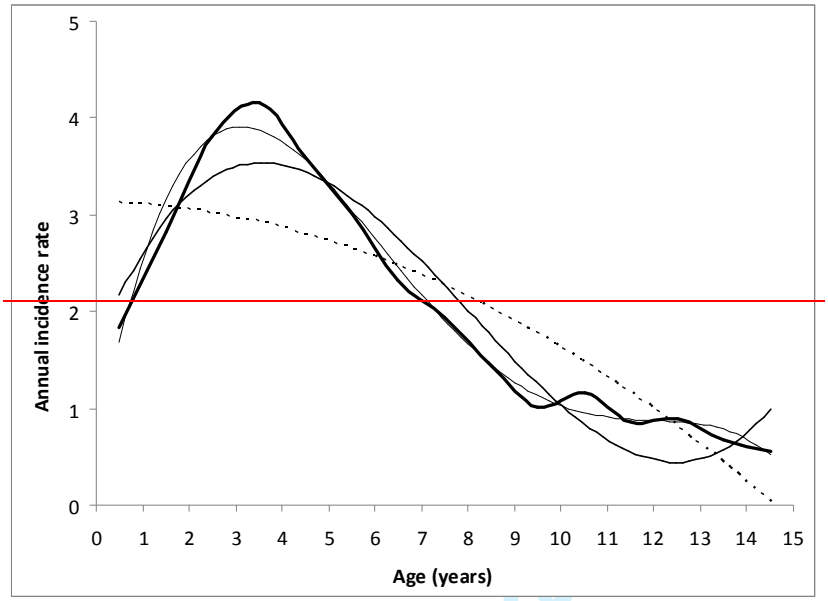
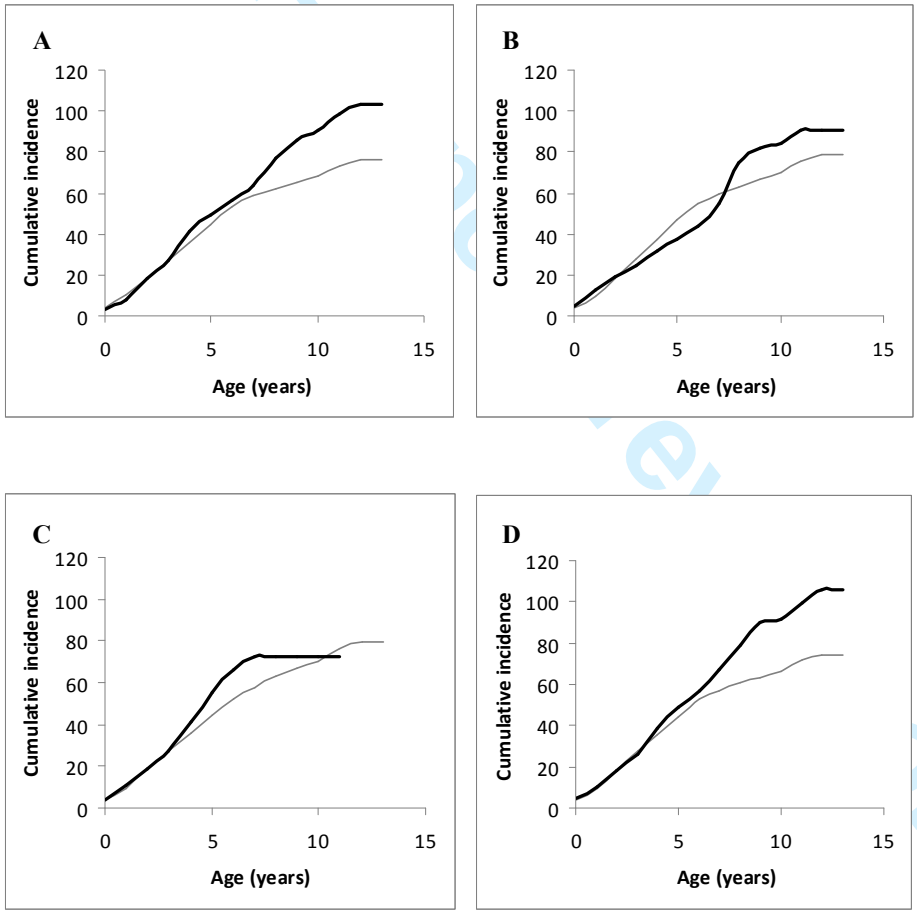


Figure 2 Mean cumulative number of *P. falciparum* clinical episodes with age for the (A) Asthma, (B) Rhinoconjunctivitis and (C) Atopic dermatitis classes and overall Atopy class (D) (bold lines) compared to individuals without symptoms of each respective allergy type (thin lines). In all cases moderate and severe classes are combined and compared to individuals without allergy symptoms. Note there are no children older than 11 years of age with Atopic dermatitis.



INSTITUT PASTEUR	Unit of Functional Genetics of Infectious Disease	Coding :	
	ENREGISTREMENT	03/MM/YYYY	Version : 1
	ALLERGY MODIFIED ISAAC QUESTIONNAIRE		

Place of study	***Research Institute responsible***
Technician X	*Name of Institute*
Technician X	*Principle investigator*
Technician X	*Project manager*
☎ +	☎ +

IDENTIFICATION

Validation zone

Date of questionnaire : |__|__| |__|__| |__|__|__|__| dd/*mm/yyyy* — |__|__| |__|__| |__|__|__|__| DTEQUE

Name of investigator :……

Name of study supervisor :……

Child :

First and last name of child :

Identification code of child : — |__|__| |__|__| IDENF

Date of birth : — |__|__| |__|__| |__|__|__|__| DOB

Sex : — |__| SEXE

Village/town : — |__| VILLAGE

Identification code of Questionnaire : — |__| |__|__| |__| |__|__||__|__| IDQUES

Identification code of father : — |__|__| |__|__| IDFA

Identification code of mother : — |__|__| |__|__| IDMO

Weight : |__|__|,|__| **(kg)** — |__|__|,|__| (Kg) WEIGHT

Height : |__|__|__|,|__| **(cm)** — |__|__|__|,|__| (cm) HEIGHT

Mid Upper Arm Circumference: |__|__| **(cm)** — |__|__| (cm) MUAC

Person questioned :

Last name of person questioned :…… — …… NAMEPQ

First name of person questioned :…… — …… LASTNAMEPQ

Relationship to child : Mother ☐1 Father ☐2 Brother/Sister ☐3 Grand-parents ☐4 Other ☐5 — |__| RELCHILD

If other, define : …… — …… OTHERREL

FACTORS PREDISPOSING ATOPY

First days of life : Consultation of health records of child and maternity records of mother

1. How much did your child weigh at birth ?

<1500 g ☐1 — [2500-3500[g ☐4

[1500-2000[g ☐2 — ≥ 3500 g ☐5

[2000-2500[g ☐3 — Record not found/NSP ☐9

|__| BIRTHWEIGH

2. Until what age did your child breastfeed (**exclusively or mixed**) ?
Corresponds to the age of weaning of child

< 6 months ☐1 6 – 12 mths ☐2 12 – 24 mths ☐3 >24 mths ☐4 NSP ☐9

|__| AGEWEAN

3. Until what age did your child breastfeed **exclusively** without ever taking other aliments (fruits, vegetables, rice, meat, fish, etc.) or liquids (powdered milk, cow or goats milk, fruit juice, water, etc.) ? < 6 months ☐$_1$ 6 – 12 mths ☐$_2$ 12 – 24 mths ☐$_3$ NSP ☐$_9$		|__|	AGEBREAST
Illness and vaccination : Consultation of health records of child			
1. Has your child enfant had the following illnesses?			
Malaria :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	MALAR
Tuberculosis treated :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	TUBTRT
Helminths (oxyures, ascaris, taenia, etc.) :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	HEMINTH
Amoeba :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	AMOEBA
Measles :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	MEASLES
2. Against what illnesses is you child vaccinated?			
Yellow fever :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACFJ
Hepatitis B :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACHEPB
Measles :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACMEASLE
Mumps :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACMUMPS
Rubella :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACRUBEL
Tuberculosis/BCG :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACTUB
Diphtheria/Tetanus/Pertussis/Poliomyelitis :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACDTCP
Typhoid :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACTY
Meningitis :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACMENIN
Haemophilus influenzae type B (HiB) :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACHIB
Habitation :			
1. Which of these animals / insects can be found in the **rooms** where your child lives (today and/or during his first year of life) ?			
Dogs in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	DOGTODAY
Dogs in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	DOG01YR
Cats in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	CATTODAY
Cats in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	CAT01YR
Sheep in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	SHEEPTODAY
Sheep in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	SHEEP01YR
Goats in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	GOATODAY
Goats in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	GOA01YR
Chicken, ducks in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	CHICTODAY
Chicken, ducks in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	CHIC01YR
Rodents (rats, mice, etc.) in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	RODTODAY
Rodents (rats, mice, etc.) in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	ROD01YR
Cockroaches in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	COCTODAY
Cockroaches in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	COC01YR
Other in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	OTHTODAY
Other in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	OTH01YR
If Others, define :……		……	NAMEOTH
2. Which of these animals could be in **contact** with your child **at least once per week**			

(today and/or during his first year of life) ?

Contact with Dogs today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CDOGTODAY
Contact with Dogs 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CDOG01YR
Contact with Cats today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCATODAY
Contact with Cats 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCAT01YR
Contact with Sheep today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CSHEEPTODAY
Contact with Sheep 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CSHEEP01YR
Contact with Goats today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CGOATODAY
Contact with Goats 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CGOA01YR
Contact with Chicken, Ducks today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCHICTODAY
Contact with Chicken, Ducks 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCHIC01YR
Contact with donkeys, horses today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CHORSTODAY
Contact with donkeys, horses 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CHORS01YR
Contact with Cows, zébus today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCOWTODAY
Contact with Cows, zébus 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCOW01YR
Contact with Rodents (rats, mice, etc.) today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CRODTODAY
Contact with Rodents (rats, mice, etc.) 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CROD01YR
Contact with Other today :	☐0 No ☐1 Yes ☐9 NSP	|__|	COTHTODAY
Contact with Other 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	COTH01YR
If Others, define :……		……	NAMEOTHC

3. Which of these aliments are usually stocked in the rooms where your child lives ?

Millet kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	MIL
Sorghum kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	SORG
Maize kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	MAIZ
Rice kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	RICE
Wheat kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	WHEA
Biscuits, pasta kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	BISCUI
Manioc (root, flour) kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	MANIOC
Cashew nut, ground nut kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	NUTP
Curdled milk kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	MILKCURD
Dried leaves (mint, quinquiliba, baobab, etc.) :	☐0 No ☐1 Yes ☐9 NSP	|__|	LEAF
Other aliments kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	OTHALIM
If Others, define :……		……	NAMEOTHAL

What is the type of roofing of the rooms where your child lives (today and during the first year of life) ?

Corrugated metal roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	RMETTODAY
Corrugated metal roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RMET01YR
Thatched roof today:	☐0 No ☐1 Yes ☐9 NSP	|__|	RTHATDAY
Thatched roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RTHAT01YR
Wooden roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	RWOOTODAY
Wooden roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RWOO01YR
Cement roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	RCEMTODAY
Cement roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RCEM01YR
Plaster roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	RPLATODAY
Plaster roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RPLA01YR
Other type of roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	ROTHTODAY

Other type of roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| ROTH01YR
If other, define :.....		..... NAMEOTHR

4. Which of these objects are in the room where your child sleeps (today and during the first year of life) ?

Mattress in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| MATRTODAY
Mattress in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| MATR01YR
Bednet in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| BEDNTODAY
Bednet in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| BEDN01YR
Wardrobe in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| WARDTODAY
Wardrobe in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| WARD01YR
Chest, trunk in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| CHESTODAY
Chest, trunk in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| CHES01YR
Table in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| TABPTODAY
Table in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| TABP01YR
Chair in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| CHPTODAY
Chair in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| CHA01YR
Carpet, rug in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| CARPTODAY
Carpet, rug in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| CARP01YR
Matting in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| MATPTODAY
Matting in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| MATP01YR
Curtains in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| CURTTODAY
Curtains in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| CURT01YR
Malagasy fire in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| FIRTODAY
Malagasy fire in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| FIR01YR
Other objects in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| OTHOBTODAY
Other objects in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| OTHOB01YR
If other, define :.....		..... NAMEOTHOB

5. On what type of bedding does your child sleep (today and during the first year of life) ?

Foam mattress today :	☐0 No ☐1 Yes ☐9 NSP	|__| FMATRTODAY
Foam mattress 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| FMATR01YR
Plant fibre mattress (straw, etc.) today :	☐0 No ☐1 Yes ☐9 NSP	|__| PLFMATRTODAY
Plant fibre mattress (straw, etc.) 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| PLFMATR01YR
Wool mattress today :	☐0 No ☐1 Yes ☐9 NSP	|__| WOMATRTODAY
Wool mattress 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| WOMATR01YR
Feather mattress today :	☐0 No ☐1 Yes ☐9 NSP	|__| FEATHMTODAY
Feather mattress 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| FEATHM01YR
Plastic matting today :	☐0 No ☐1 Yes ☐9 NSP	|__| PLMATTODAY
Plastic matting 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| PLMAT01YR
Plant fibre matting (straw, etc.) today :	☐0 No ☐1 Yes ☐9 NSP	|__| PLFMATTODAY
Plant fibre matting (straw, etc.) 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| PLFMAT01YR
Other type of bedding today :	☐0 No ☐1 Yes ☐9 NSP	|__| OTHBEDTODAY
Other type of bedding 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| OTHBED01YR
If other, define :.....		..... NOMAUTLI

6. Does your child sleep on a pillow ? ☐0 No ☐1 Yes ☐9 NSP \|__\| PILLOW

If **No**, go to question **8**

If **Yes**, what type of pillow is it ?

Foam :	☐0 No ☐1 Yes ☐9 NSP	|__|	PILLF
Synthetic fibres:	☐0 No ☐1 Yes ☐9 NSP	|__|	PILLSYN
Plant fibres (straw, etc.) :	☐0 No ☐1 Yes ☐9 NSP	|__|	PILLPLF
Feather :	☐0 No ☐1 Yes ☐9 NSP	|__|	PILLFEATH
Other type of pillow :	☐0 No ☐1 Yes ☐9 NSP	|__|	OTHPILL
If other, define :.....		.....	NAMEOTHPILL

7. Do people smoke in the room where your child lives ?

Today :	☐0 No ☐1 Yes ☐9 NSP	|__|	SMOKTODAY
From 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	SMOK01YR
During the pregnancy of the mother :	☐0 No ☐1 Yes ☐9 NSP	|__|	SMOKPREG

8. What type of heating and lighting are used in the rooms where your child lives ?

Heating and lighting by charcoal :	☐0 No ☐1 Yes ☐9 NSP	|__|	CHELCHAR
Heating and lighting by wood :	☐0 No ☐1 Yes ☐9 NSP	|__|	CHELWOO
Lighting by candle :	☐0 No ☐1 Yes ☐9 NSP	|__|	LCAND
Lighting by petrol lamp :	☐0 No ☐1 Yes ☐9 NSP	|__|	LLAMP
Lighting by flash light :	☐0 No ☐1 Yes ☐9 NSP	|__|	LTORCH
Lighting by solar :	☐0 No ☐1 Yes ☐9 NSP	|__|	LSOLAR
Other types of heating and lighting:	☐0 No ☐1 Yes ☐9 NSP	|__|	OTHHEL
If other, define :.....		.....	NAMEOTHHEL

9. Which of the following products are used or stocked in the rooms where you child lives ?

Insecticide (type Yotox, spirales, etc.) :	☐0 No ☐1 Yes ☐9 NSP	|__|	INSECTIC
Deodorants (aerosols) :	☐0 No ☐1 Yes ☐9 NSP	|__|	DEODORA
Incense :	☐0 No ☐1 Yes ☐9 NSP	|__|	INCENSE
Detergents (type Cotol, etc.) :	☐0 No ☐1 Yes ☐9 NSP	|__|	DETERGEN
Petrol, diesel :	☐0 No ☐1 Yes ☐9 NSP	|__|	PETROL
Other types of products :	☐0 No ☐1 Yes ☐9 NSP	|__|	OTHPROD
If other, define :.....		.....	NAMEOTHPR

Diet :

1. Has your child had **diarrhoea without fever** or abdominal pains (colic)

following introduction of non-maternal milk in his diet (cow or goat's milk, milk powder) :	☐0 No ☐1 Yes ☐9 NSP	|__|	DIARINT
after a few months of consuming **non-maternal** (cow or goat's milk, milk powder) :	☐0 No ☐1 Yes ☐9 NSP	|__|	DIARMONTH

2. Currently, how many times, on average, does your child eat the following aliments ?

The consumption of certain aliments is seasonal.

Meat :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMEAT
Fish :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSFISH
Egg :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSEGG
Milk (liquid, powder, curdled) :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMILK
Banana :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSBANA
Mango :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMANG
Melon :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMELON

Orange, lime :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSORAN
Potatoes, sweet potatoes :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSPOT
Vegetables :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSVEG
Millet :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMIL
Sorghum :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSSORG
Maize :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMAIS
Rice :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSRICE
Wheat (bread, pasta) :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSWHEA
Nuts (Cashew, ground nut) :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSNUT
Prawns, dried oysters :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSPRAWN
Flavouring cubes Maggi :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSCUBE
Other :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	OTHALCON
	If other, define :.....	.....	NAMEOTHAL

HISTORICAL SYMPTOMATOLOGY OF ALLERGIC REACTIONS

Asthma :

1. Has a doctor or nurse **already** said that you child has asthma ?

☐0 No ☐1 Yes ☐9 NSP — \|__\| ASTHMA

2. Has your child already breathed noisily or had whistling in his chest whilst breathing

☐0 No ☐1 Yes ☐9 NSP

If **No**, go directly to question **6**

\|__\| WHISTLING

3. During his first two years of life, has your child already breathed noisily or had whistling in his chest whilst breathing ?

☐0 No ☐1 Yes ☐9 NSP — \|__\| WHISTL2YR

If **No**, go directly to question **6**

If **Yes**, how many times (before 2 years of age) ?

☐1 1time ☐2 2times ☐3 ≥3times ☐9 NSP — \|__\| NBWHIS2YR

Between the last two **ramadans**, has your child already breathed noisily or had whistling in his chest whilst breathing ?

☐0 No ☐1 Yes ☐9 NSP — \|__\| WHISTL2RA

If **No**, go directly to question **5**

If **Yes**, at which moment of the year ?

Rainy season :	☐0 No ☐1 Yes ☐9 NSP	|__| WHISTLRS
Dry season :	☐0 No ☐1 Yes ☐9 NSP	|__| WHISTLDS
Harvest time :	☐0 No ☐1 Yes ☐9 NSP	|__| WHISTLHT

Has the noisy breathing of your child been such that it has prevented him from talking normally?

☐0 No ☐1 Yes ☐9 NSP — \|__\| PREVTALK

Has your child already had a rasping cough at night that prevents him from sleeping normally ?

☐0 No ☐1 Yes ☐9 NSP — \|__\| TOUSECHE

Rhinitis and allergic conjunctivitis;

1. Has your child **already had** problems of a runny nose, **or** a sensation of a blocked nose, **or** an itchy nose, **or** sneezing, or loss of the sense of smell **for more than a week,**

irrespective of the frequency of these episodes? ☐0 No ☐1 Yes ☐9 NSP	|__|	RHIN1WEEK
2. Has your child **already had** problems of a runny nose, **or** a sensation of a blocked nose, **or** an itchy nose, **or** sneezing, or loss of the sense of smell **more than 5 times in one year,** irrespective of the frequency of these episodes? ☐0 No ☐1 Yes ☐9 NSP	|__|	RHIN5FAN
Between the **last two ramadans**, has your child **already had** problems of a runny nose, **or** a sensation of a blocked nose, **or** an itchy nose, **or** sneezing, or loss of the sense of smell ? ☐0 No ☐1 Yes ☐9 NSP	|__|	RHIN2RAM
If **No**, go to question **4**		
If **Yes**, at what moment of the year ?		
Rainy season : ☐0 No ☐1 Yes ☐9 NSP	|__|	RHINRS
Dry season : ☐0 No ☐1 Yes ☐9 NSP	|__|	RHINDS
Harvest time : ☐0 No ☐1 Yes ☐9 NSP	|__|	RHINHT
3. Has your child **already had** watery eyes, **or** itchy eyes, **or** an allergic limbo-conjonctivitis? ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJALER
If **No**, go directly to question **1** in the section **Eczema**		
Has your child had, between the **last two ramadans**, watery eyes, **or** itchy eyes, **or** an allergic limbo-conjonctivitis? ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJ2RAM
If **No**, go directly to question **5**		
If **Yes**, at what moment of the year ?		
Rainy season : ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJRS
Dry season : ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJDS
Harvest time : ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJHT
Eczéma :		
Has your child **already had** skin problems with dry patches or seeping cracked patches and itching ? ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEMA
If **No**, **the questionnaire has finished.**		
Between the **last two ramadans**, has your child had skin problems with dry patches or seeping cracked patches and itching ?? ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZE2RAM
If **No**, go directly to question **3**		
If **Yes**, at what moment of the year ?		
Rainy season : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEMARS
Dry season : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEMADS
Harvest time : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEMAHT
1. Have these skin problems affected different parts of the body of your child ?		
Scalp : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZESCALP
Face : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEFAC
Around the eyes and ears : ☐0 No ☐1 Yes ☐9 NSP		
Armpits : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEEYEEAR

Elbow :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEARMPIT
Hands :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEELBOW
Under the buttocks:	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEHAND
Groin :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEBUTT
Behind the knee :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEGROIN
Feet :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEKNEE
Other part of body :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEFEET
		|__| ECZEOTH

What age did your child have when these skin problems of dry patches, weeping cracked patches or itching appear for the **first time**?

☐1 < 2yr ☐2 2 - 4 yr ☐3 ≥ 5yr

\|__\| AGECZEMA

Have your child's skin problems ever been sufficiently important to prevent him from sleeping correctly or waking him up during the night ?

☐0 No ☐1 Yes ☐9 NSP

\|__\| IRRECZEM

Comments : Note with reference to which questions these comments apply

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STROBE Statement—checklist of items included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Asthma and atopic dermatitis are associated with increased risk of clinical Plasmodium falciparum malaria

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Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium falciparum* malaria

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Article summary

Article focus

- Genetic studies suggest a link between susceptibility to allergy and malaria in Africa
- We hypothesize that atopy increases susceptibility to malaria

Key messages

- Results demonstrate an association between asthma, atopic dermatitis and susceptibility to clinical *P. falciparum* episodes.
- Genetic pre-disposition to asthma or atopic dermatitis impairs the acquisition of clinical immunity to malaria.
- Administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Strengths and limitations

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association.

Abstract

Objectives: To assess the impact of atopy and allergy on the risk of clinical malaria.

Design: A clinical and immunological allergy cross-sectional survey in a birth cohort of 175 children from 1 month to 14 years of age followed for up to 15 years in a longitudinal open cohort study of malaria in Senegal. Malaria incidence data were available for 143 of these children (aged 4 months to 14 years of age) for up to 15 years. Mixed model regression analysis was used to determine the impact of allergy status on malaria incidence, adjusting for age, sickle cell trait and force of infection.

Main outcome measures: Asthma, allergic rhinoconjunctivitis and atopic dermatitis status, the number of clinical *Plasmodium falciparum* malaria episodes since birth and associated parasite density.

Results: Twelve percent of the children were classified as asthmatic and ten percent as having atopic dermatitis. These groups had respectively a two-fold (OR 2.12 95% confidence intervals 1.46 to 3.08; P= 8 $x10^{-5}$) and three-fold (OR 3.15, 1.56 to 6.33; P= 1.3 $x10^{-3}$) increase in the risk of clinical *P. falciparum* malaria once older than the age of peak incidence of clinical malaria (3 to 4 years of age). They also presented with higher *P. falciparum* parasite densities (Asthma: mean 105.3 parasites/μL ±SE 41.0 *vs*. 51.3 ±9.7; P= 6.2 $x10^{-3}$; Atopic dermatitis: 135.4 ±70.7 *vs*. 52.3 ±11.0; P=0.014). There was no effect of allergy on the number of non-malaria clinical presentations. Individuals with allergic rhinoconjunctivitis did not have an increased risk of clinical malaria nor any difference in parasite densities.

Conclusion: These results demonstrate that asthma and atopic dermatitis delay the development of clinical immunity to *P. falciparum*. Despite the encouraging decrease in malaria incidence rates in Africa, a significant concern is the extent to which the increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines, administration to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Introduction

The World Allergy Organization estimates that 40% of the world's population is concerned by allergic diseases.[1] In developing countries where *Plasmodium falciparum* malaria is endemic, prevalence of allergy is significantly lower, but is on the increase.[2] Orientation of the immune response towards a Th1 profile is crucial for immunity to intracellular pathogens,[3] whereas orientation towards a Th2 profile drives immunity to extracellular pathogens and antigens resulting in class switching giving rise to IgE-producing B cells.[4] An important role of the Th1/Th2 balance in the development of clinical malaria following infection by *P. falciparum* has been suggested by numerous studies.[5-7] It has been suggested that the Th2 bias induced by *P. falciparum* may exacerbate allergy.[8] Likewise, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*. However, the interplay between infectious agents and allergy is unclear. On the one hand, for example, severe respiratory syncytial virus infection in infants increased the risk of allergic rhinoconjunctivitis and allergic asthma.[9,10] On the other hand, measles,[11] hepatitis A[12] and tuberculosis[13] seemingly reduce atopy. Although, an atopic condition can increase incidence of disease, such as the case for the skin commensal *Staphylococcus aureus* in patients with atopic dermatitis,[14] an atopic tendency *per se* does not generally lead to increased illness from infectious agents.

Genome wide studies have identified chromosomal regions linked to clinical malaria, all of which overlap with those previously identified to be involved in atopic dermatitis, asthma, atopy and IgE levels,[15-17] suggesting that common mechanisms may be involved in both pathologies.[18] Chromosomal region 5q31 that has been repeatedly shown to be associated with control of parasite density and contains a cluster of cytokines, among which IL12B has been previously associated with psoriasis.[19] The other regions, 13q13-q22, 5p15-p13 and 12q21-q23, contain genes involved in innate immunity, notably the interleukin 7 receptor, and several involved in tumour necrosis factor synthesis [C1q and tumour necrosis factor related protein 3 (C1QTNF3)] and a gene involved in the complement system (C9). [18]

Several additional lines of evidence support the concept that susceptibility to malaria and atopy may be related to similar immunological defects. In Ethiopia, a history of malaria was associated with atopy.[20] A mouse model for human atopic disease was found to be very susceptible to murine malaria and a major locus for atopic disease mapped close to the

region controlling parasite density.[21] This region contains several candidate genes that have effects on T-cell function.[21]

Moreover, a direct effect of histamine in the malaria pathogenesis has been found using genetic and pharmacological approaches[22] and increased levels of histamine are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[23,24]

To test the hypothesis that allergy impacts upon clinical *P. falciparum* malaria, we performed a clinical allergy cross-sectional study in the family-based longitudinal cohort from Senegal previously used for the genome linkage study[18] and analysed the impact of asthma, atopic dermatitis, allergic rhinoconjunctivitis on the incidence of clinical *P. falciparum* episodes and the maximum parasite density during each episode.

Methods

Population and outcome data

The malaria research program conducted in Dielmo village in Senegal has been ongoing since 1990 as described elsewhere.[25] In brief, between 1990 and 2008, a longitudinal study involving the inhabitants of the village of Dielmo, Senegal, was carried out to identify all episodes of fever. The study design included daily medical surveillance with systematic blood testing of individuals with fever and examination of 200 oil-immersion fields on a thick blood film for malaria parasites (about 0.5 µL of blood). Each individual was given a unique identification code and details of family ties, occupation, and precise place of residence were recorded on detailed maps of each household with the location of each bedroom. All households were visited daily, absenteeism recorded, and the presence of fever or other symptoms assessed. We systematically recorded body temperature at home three times a week (every second day) in children younger than 5 years, and in older children and adults in cases of suspected fever or fever-related symptoms. In cases of fever or other symptoms, blood testing was done at the dispensary by finger prick, and we provided detailed medical examination and specific treatment. Parasitologically confirmed clinical malaria episodes were treated according to national guidelines. From 1990 to 2008, four different drug regimens were implemented: Quinine from 1990 to 1994, Chloroquine from 1995 to 2003,

Fansidar (sulfadoxine-pyrimethamine) from 2004 to mid-2006 and Artemisinin-based combination therapy (ACT; Amodiaquine- sulfadoxine-pyrimethamine) from mid-2006 to 2008.

Parasite positivity was established as follows. Thick blood films were prepared and stained by 3% Giemsa stain. Blood films were examined under an oil immersion objective at x1000 magnification by the trained laboratory technicians and 200 thick film fields were examined to count the number of asexual and gametocyte parasite stages. Asexual parasite densities (per µL) were calculated by establishing the ratio of parasites to white blood cells and then multiplying the parasite count by 8,000, the average white blood cell count per µL of blood.

Malaria transmission in Dielmo is intense and perennial. We conducted a cross-sectional survey to estimate the prevalence of symptoms related to allergic diseases among 175 children aged from 1 month to 14 years old who were born during the malaria research program.

Both the longitudinal and cross-sectional surveys were approved by the Ministry of Health of Senegal. Informed consent of the volunteers is renewed every year. More specifically for the cross-sectional survey, after informing about the procedures and the purpose of the study, written informed consent was obtained from parents or guardians of children either by signature or by thumbprint on a voluntary consent form written in both French and Wolof, the main local language. Consent was obtained in the presence of the school director, an independent witness.

The family structure (pedigree) was available after a demographic census performed for every volunteer at his adhesion in the project. A verbal interview of mothers or key representatives of the household was used to obtain information on genetic relationships between studied individuals, their children, their parents, and to identify genetic links among the population. The total pedigree comprised 828 individuals, including absent or dead relatives, composed of ten independent families that can be sub-divided into 206 nuclear families (father – mother couples with at least one child) with an average of 3.6 children each. Genetically related nuclear families occur because of multiple marriages and marriages among related individuals. Previous typing with microsatellites has enabled the construction of a pedigree based on Identity-by-Descent using MERLIN.[18,26] The mean coefficient of inbreeding is 0.0008. Newborns since this original genetic analysis were added

to the family of the parents in question. The 143 children, with both allergy and malaria data, belonged to 61 nuclear families and comprised 30 singletons, 102 siblings and 11 half-sibs (yielding 55 half-sib pairs). The mean genetic relatedness (by pedigree) of the 143 children is 0.0114 (range: 0.0013 to 0.022).

P. falciparum *clinical episodes*

P. falciparum malaria clinical episode phenotypes analysed were: (i) clinical *P. falciparum* infections treated with anti-malarial therapy and (ii) the highest parasite density during the *P. falciparum* clinical episode. A clinical *P. falciparum* episode was defined as a clinical presentation with fever (axillary temperature ≥ 37.5°C) and/or other clinical signs suggestive of malaria associated with a thick blood smear positive for *P. falciparum* and that was treated with anti-malarial therapy. Repeated clinical malaria presentations within 15 consecutive days were not considered to be independent and were excluded from the analyses, unless there was a negative thick blood smear between two clinical presentations. We also excluded observations in any trimester for which the individual was not present for at least one third of the time.

We calculated the quarterly incidence rate of clinical *P. falciparum* episodes in children below the age of 15 years as the ratio of the total number of clinical *P. falciparum* episodes during the trimester divided by the total number of person-trimesters surveyed. Incidence rate is expressed as cases per 100 person-trimesters (see Supplementary Figure S1). This rate was used in the analysis to approximate the force of infection (exposure level) within the targeted population at the time of a given clinical *P. falciparum* episode.

The total number of clinical presentations per trimester that were not attributable to *P. falciparum* was tabulated. Repeated non-malaria presentations within seven consecutive days were not considered to be independent and were excluded.

Allergic diseases and atopic status

The International Study of Asthma and Allergies in Childhood (ISAAC) diagnostic criteria have been shown to be reproducible, adequate and able to discriminate children with allergic diseases in different areas of the world.[2] The standardized ISAAC questionnaire originally written in English was translated into French in compliance with ISAAC guidelines[27], adapting it to the usual local customs following advice from local clinicians and paediatric

allergologists (Acknowledgements and Technical Appendix). The adequacy and reliability of the translated questionnaire had been previously confirmed by a pilot study on 30 randomly selected children in the same community. The questionnaire was completed by specially trained health workers during an oral interview conducted in Wolof with children and their mothers or guardians.

To assess the prevalence of allergic diseases in children, we used the positive and negative predictive values of the ISAAC questionnaire diagnosis criteria developed for subtropical countries.[28] Each question was scored according to the medical diagnosis of paediatricians and paediatric allergologists. Positive or negative answers were thus graded on the basis of symptom sensitivity, specificity, frequency, location or early onset. For each allergic disease, three categories of symptom severity, *severe*, *moderate*, and *none*, were defined as follows:

Asthma – severe symptoms if the child had "wheezing or whistling in the chest before the age of two years" and "more than three times" *or* severe enough to "limit his/her speech"; *moderate* symptoms if the child had "wheezing or whistling in the chest before the age of two years" and "in the past 12 months"; and *none* otherwise.

Allergic rhinoconjunctivitis – severe symptoms if the child had "sneezing, runny or stuffy nose in the past 12 months" and "more than five times a year", and "itchy, watery eyes or tropical endemic limboconjunctivitis (TELC) in the past 12 months"; *moderate* symptoms if the child had "sneezing, runny or stuffy nose in the past 12 months", and "itchy, watery eyes or TELC in the past 12 months"; and *none* otherwise.

Atopic dermatitis – severe symptoms if the child had "scaly or exudating, crusted and pruritic patches in the past 12 months" and "affecting any of the following characteristic areas: face, around the ears or eyes, folds of armpits or elbows or groin, behind the knees, under the buttocks", and "onset of symptoms before the age of two years"; *moderate* symptoms if the child had "scaly or exudating, crusted and pruritic patches in the past 12 months" and "affecting any of characteristic areas (see above)", and "onset of symptoms before the age of four years"; and *none* otherwise.

The inter-relationships between variables reflecting the severity of symptoms of the three allergic diseases were used to identify children at high risk of atopy. The *high probability* group was defined by the prevalence of at least one of any *severe* symptoms or two of any

moderate symptoms. The *probable* group was defined as those with *moderate* symptoms from one of the three allergic diseases and remaining children were classified in the *unlikely* group.

Helminths

Helminthic infections are common in this region and are known to modify the clinical course and outcome of both allergic diseases and malaria.[29,30] We therefore carried out a helminth survey for 91 individuals present during the cross-sectional survey. Diagnosis was performed by stool examination by microscope and by the Kato technique to search for the presence of *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*), whipworm (*Trichuris trichiuria*), *Schistosoma mansoni*, and *Strongyloides stercoralis*. Examination for pinworms (*Enterobius vermicularis*) was performed by the anal scotch-test. An anti-helminthic treatment was proposed for all infested individuals.

Immunoglobulin E titres

Specific IgE titres were measured by ELISA as previously described.[31] A panel of allergens of potential pertinence to the three classes of allergy was used: (i) Salivary gland extracts (SGE) of two mosquito species present in the study cohorts, *Aedes aegypti* and *Anopheles gambiae sensu stricto*, and (ii) *P. falciparum* parasite extract were prepared as previously described[31]; (iii) House dust mite spp. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*; (iv) a mix of pollen allergens from five ubiquitous graminae spp. [Cock's-foot (*Dactylis glomerata*), Timothy grass (*Phleum pratense*), Sweet Vernal grass (*Anthoxanthum odoratum*), Perennial ryegrass (*Lolium perenne*), Kentucky Bluegrass (*Poa pratensis*)] (all from Stallergenes, France).

Statistical analysis

Statistical analyses were performed using R version 2.12.0 (The R Foundation for Statistical Computing, Vienna, Austria). To address the effect of allergic status on the risk of clinical *P. falciparum* episodes, we performed Generalized Linear Mixed Models (GLMM) extended to pedigree data using the *pedigreemm* package for R to account for the non-independence of individuals because of family relationships, shared house and for repeated measures from the same individual (Technical Appendix). Correlated individual effects due to familial relationships were taken into account by using the pedigree-based genetic relatedness

matrix that contains the genetic covariance among all pairs of individuals in the study cohort and is calculated using the pedigree information.[32] Shared house and repeated measures from the same individual were modelled as random effects. All random effects were assumed to be normally distributed, and conditional on these random effects, the dependent variable had: (i) a Binomial distribution when the studied phenotype was the occurrence of a clinical *P. falciparum* episode treated with anti-malarial therapy during a trimester, (ii) a Gaussian distribution when the studied phenotype was the logarithm of the maximum parasite density during a given clinical *P. falciparum* episode, and (iii) a Poisson distribution when the studied phenotype was the number of non-malaria episodes per trimester. The effects of allergy disease classes on these dependent variables were modelled as fixed effects. Allergy classes were reduced to two levels, *Severe* or *moderate vs*. *none* for analyses of asthma, atopic dermatitis and allergic rhinoconjunctivitis and *high probability vs*. *probable* and *unlikely* for atopic tendency. Co-variables included sickle cell trait[31], gender, number of days present on site during the trimester, trimestrial incidence of *P. falciparum* and age. Age was initially analysed as a continuous covariate. To assess the age-specific effect of allergy, age was categorised into two levels (<3.5 years of age and ≥3.5 years of age, based on the age of peak clinical incidence) and allergy class was nested within age class. The age threshold was varied from 1.5 years to 5.5 years of age and the data re-analysed to assess at which age there was the strongest effect. The association of allergy classes with IgE levels was analysed by box-cox transforming the data and fitting a GLMM with a normal distribution.

Results

Of the 205 eligible children aged under 15 years involved in the family-based longitudinal study, 175 (85.4 %) participated in the cross-sectional survey to assess the prevalence of related symptoms of allergic diseases. All eligible children present at the time of the survey were included; no explicit refusal to participate was recorded. The study cohort was aged from 1 month to 14 years 11 months. The sex-ratio (male/female) was 0.94.

From 1994 until 2008, 143 of the children participating in the cross-sectional survey were present for at least 31 days in any trimester during the study period generating a total of

3,093 person-trimesters of presence (Supplementary Table S1). There were 2,065 treated *P. falciparum* clinical episodes (median 11, range 0-47)(Supplementary Table S2). The age peak of incidence of *P. falciparum* episodes occurred at 3 to 4 years of age (Figure 1). There were 1,868 non-malaria episodes (median 12, range 0-37) (Table S2). These non-malaria clinical presentations were associated with headache (38 %), chills (32 %), cough (13 %), vomiting (11 %) and diarrhoea (6 %).

The prevalence of moderate or severe asthma symptoms was respectively 2.3 % and 10.3 % (Table 1). The prevalence of moderate or severe allergic rhinoconjunctivitis symptoms was respectively 6.3 % and 10.3 %. The prevalence of moderate or severe atopic dermatitis symptoms was respectively 6.3 % and 2.9 %. On the basis of symptom severity, an atopic tendency was estimated to be unlikely for 68.0 %, probable for 9.1 % and highly probable for 22.9 % of the 175 children. The frequency of each allergy class in children for whom malaria data were available is shown in Table S1.

The risk of treated clinical *P. falciparum* infections was higher for children with high probability of atopy (OR 1.65, 95% confidence intervals 1.20 to 2.26; P=0.002) (Table 2), after adjusting for age, sickle cell trait and the exposure level. Gender was not found to be significant. Analysing the impact of atopy in children younger and older than the peak age of clinical incidence (3 to 4 years old), revealed that atopy increased the risk of *P. falciparum* episodes in children at an age greater than 3.5 years (OR 2.02, 1.39 to 2.93; $P=2x10^{-4}$), but not in children of age prior to the peak clinical incidence (OR 1.38, 0.92 to 2.08; P=0.124) (Table 2). This increased risk resulted in an ever increasing cumulative number of *P. falciparum* episodes with age beyond that of peak clinical incidence (Figure 2. See supplementary Figure S2 for model predictions for comparison).

Analysis by allergy category revealed that asthma (severe or moderate) increases the risk of *P. falciparum* episodes (OR 2.12, 1.46 to 3.08; $P= 8 x10^{-5}$) and this again only in children of age greater than 3.5 years old (OR 2.33, 1.50 to 3.61; $P= 1.5 x10^{-4}$). Atopic dermatitis increased the risk of clinical malaria in children older (OR 3.15, 1.56 to 6.33; $P= 1.3 x10^{-3}$) but not younger than 3.5 years of age (Table 2). Allergic rhinoconjunctivitis was not associated with increased risk of clinical malaria at any age (Table 2). The impact of atopy, asthma and atopic dermatitis can be clearly seen in the ever-increasing number of cumulative *P. falciparum* episodes beyond the age of the onset of clinical immunity in the population, 3.5

years of age (Figure 2). There is no difference in the number of clinical malaria episodes prior to this age in individuals with or without an allergic condition. Analysis using different age thresholds (from 1.5 to 5.5 years of age) revealed similar OR for thresholds of 2.5, 3.5 and 4.5 years of age. The maximum OR for increased malaria occurred in children older than 4.5 years of age and with atopy or atopic dermatitis, whereas for the asthma group it occurred in children after 3.5 years of age (Supplementary Table S3).

There was no impact of any allergic disease on the number of non-malaria episodes by trimester (Supplementary Table S4).

The impact of atopy, asthma and atopic dermatitis on the maximum *P. falciparum* parasite density during a given clinical malaria episode mirrored that of the risk of *P. falciparum* episodes. Parasite density was significantly higher for children with allergic disease older than 3.5 years of age (Table 3 and supplementary Figure S3 for residuals of the fitted model). Allergic rhinoconjunctivitis had no impact on the parasite density (Table 3). Analysis using different age thresholds yielded the same pattern as seen with the number of clinical episodes (Table S3).

Individuals with moderate or severe symptoms of atopic dermatitis had significantly higher specific IgE titres against *Ae. aegypti* (P=0.004) and *An. gambiae* SGE (P<0.001). There were no detectable specific anti-*P. falciparum* IgE. Individuals with moderate or severe symptoms of allergic rhinoconjunctivitis did not have significantly higher IgE titres against the tested graminae (P=0.28), although titres decreased with age (P=0.035). There was also no effect of asthma on IgE titres against the house dust mite spp. tested (*D. farinae* P=0.60 & *D. pteronyssinus* P=0.27).

Only five individuals were infested with helminths (two *Ancylostoma*, one *Strongyloides*, one *Trichuris* and one *Enterobius*).

Discussion

Principal findings

Establishing the allergic status of children up to the age of 15 years old followed for malaria since birth, revealed an association of asthma and atopic dermatitis with susceptibility to

clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

Strengths and weaknesses of the study

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association. In addition, although allergy diagnosis for children under 2 years of age is not considered reliable, there were only 15 individuals under 2 at the time of the allergy study of the 143 for whom malaria and allergy data were available.

Meaning of the study

Under intense malaria transmission, after repeated exposure to the parasite, children develop a clinical immunity[33], whereby they tolerate elevated parasite densities without showing clinical symptoms. In this cohort, the population mean onset of clinical immunity occurred at 3 to 4 years of age. Although clinical immunity is accompanied by a reduction in parasite density, effective anti-parasite immunity develops much more slowly[34] with individuals achieving a state of premunition, whereby they maintain low-grade parasite densities in an asymptomatic state.[35] We show here that children with clinically defined asthma or atopic dermatitis had a two to three-fold increase in the risk of presenting with *P. falciparum* malaria episodes requiring treatment once passing the age of peak clinical incidence. They also had higher parasite density during clinical episodes, suggesting a reduced ability to control parasite replication. The observed increase in clinical incidence of malaria in patients with asthma or atopic dermatitis is not likely to be the result of increased frailty of such individuals; these individuals did not come more frequently to the clinic with non-malaria symptoms. Our previous genome linkage study identifying chromosomal regions[18] associated with malaria that overlap with those previously shown to be linked to asthma/atopy suggests that there may be a shared genetic basis to these pathologies rather than any causative effect of one on the other. This is consistent with the increased susceptibility to malaria of mouse atopic models.[21]

Comparison with other studies

A previous study found that a history of malaria (yes/no) increased risk of atopic dermatitis in 306 cases compared to 426 controls as characterized using the ISAAC questionnaire.[20] The only other epidemiological study that has previously examined the link between malaria and atopy[36] also interpreted the result from the perspective of the impact of malaria on atopy. They examined the re-infection rate with *P. falciparum* over a 5-year period in 91 children that were subsequently classified as atopic or not using skin prick tests (SPT) with house dust mite antigen. Their conclusion was that, as with measles[11] and tuberculosis[13], malaria infection reduces atopy. However, the study lacked previous infection data since birth of the participating individuals and focussed on atopy as determined by SPT against a single allergen. The case-control study of atopic dermatitis risk factors cited above found no overall association between allergen skin sensitization and atopic dermatitis. We also found no evidence of increased IgE titres against house dust mites in the asthmatic or atopic dermatitis groups or against grass pollen in individuals with allergic rhinoconjunctivitis. Such differences likely reflect the different IgE reactivity profiles due to differences in allergen exposure in Africa.[37] There was no evidence of anti-parasite IgE in this cohort of children. We previously showed that circulating anti-parasite IgE titres were strongly positively correlated with anti-mosquito saliva IgE, but became undetectable following malaria exposure, potentially being bound to effector cells.[31] Only mosquito saliva, a known major local allergen, induced a specific IgE response at significantly higher titres in individuals with atopic dermatitis.

Although the immune effectors of clinical immunity are still poorly defined, there is strong evidence that acquired anti-parasite immunity is IgG-dependent[38] and cytophilic immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by opsonisation and/or by Antibody Dependent Cellular Immunity play an important role in premunition.[35] The higher parasite density during symptomatic episodes observed in the asthma group suggests impaired development of acquired immunity. Impaired acquisition of immunity to malaria in children with asthma or atopic dermatitis may stem from their imbalanced Th1/Th2 response. Indeed, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*. Dendritic cells that are oriented to a Th2 phenotype are more susceptible to orient the acquired immune response towards a Th2

profile.[39] Orientation of the immune response towards a Th2 profile by asthma or atopic dermatitis would result in a poor Th1 response (and hence development of protective IgG immunoglobulins), considered to be the dominant arm of the immune response enabling resistance to infectious disease in children.[40]

Many studies have revealed an important role of histamine, a key downstream effector molecule in allergic reaction, in the outcome of a malaria parasite infection.[22-24,,41-44] Moreover, reports indicate that components of the innate immune system, including eosinophils, basophils, and mast cells (MCs), could play important roles in the pathogenesis of malaria.[41] Increased levels of histamine in plasma and tissue, derived from basophils and MCs, notably following stimulation by IgE through the high affinity receptor FcεR1, are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[23,24] Chlorpheniramine, a HR1agonist reversed resistance to chloroquine and amodiaquine both *in vivo* and *in vitro*.[42] Moreover, astemizole, another HR1 agonist, was identified as an anti-malarial agent in a clinical drug library screen.[43] Finally, *P. falciparum* produces translationally controlled tumor protein, which is a homolog of the mammalian histamine-releasing factor that causes histamine release from human basophils.[44]

Further research

Our results provide the first birth cohort study addressing the link between malaria and allergic diseases. They contribute to a growing body of evidence that the pathologies are related. ISAAC has revealed a steady but significant increase in prevalence rates of asthma and allergic diseases in Africa. Whilst the majority of studies have focused on large cities, there is increasing urbanization throughout Africa, as well as improved access to primary health care in many areas. A key concern for ISAAC is the extent to which such societal evolution will result in an increase in allergic diseases. Increased urbanization in sub-Saharan Africa is changing the epidemiology of malaria and although resulting in a decrease in risk, will result in more severe clinical malaria in older individuals.[45,46] Moreover, a large consumption of anti-malarial drugs in the urban areas provides substantial drug pressure fostering, the selection of drug-resistant parasites. Despite the encouraging recent decrease in malaria incidence rates, even in rural areas, an additional significant concern is the extent to which such an increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines[47], administration of anti-histamines to

atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials[48] and alleviate the non-infectious consequences of atopy. Clinical intervention studies should be envisaged.

What is already known on this topic

There are several reports of the beneficial effects of anti-histamines for malaria chemoprophylaxis[22-24,47] as well as our previous work[18] showing that chromosomal regions associated with malaria are also linked to allergy and atopy.[15-17] There are two epidemiological studies showing opposite effects of malaria on atopy.[20,36]

What this study adds

Using a longitudinal malaria study birth cohort, we identified an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred only after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: The allergy study was approved by the Senegalese National Ethics committee (2009/N°46). Renewed approval of the longitudinal malaria study was obtained from the same committee (2006/N°969).

Data sharing: The allergy database will be made available on-line. The longitudinal malaria data set will be made available following discussion with the coordinators of the three Institutes that govern the dataset through contact with the corresponding author.

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Table 1 Classification of Asthma, Allergic rhinoconjunctivitis, Atopic dermatitis and overall Atopic status according to ISAAC questionnaire in children aged 0-14 from a malaria birth cohort. N is total number of children examined and n-malaria represents those for whom malaria data were recorded. F is the number of females and M the number of males.

	N (F/M)	%	n-malaria (F/M)
Asthma symptoms			
None	153 (73/80)	87.43	125 (59/66)
Moderate	4 (1/3)	2.29	4 (1/3)
Severe	18 (6/12)	10.29	14 4/10)
Rhinoconjunctivitis symptoms			
None	146 (64/82)	83.43	120 (52/68)
Moderate	11 (8/3)	6.29	9(6/3)
Severe	18 (6/12)	10.29	14 (6/8)
Atopic dermatitis symptoms			
None	159 (75/84)	90.86	128 (60/68)
Moderate	11 (1/10)	6.29	11 (1/10)
Severe	5 (4/1)	2.86	4 (3/1)
Atopic tendency			
Unlikely	119 (56/63)	68.00	97 (46/51)
Probable	16 (8/8)	9.14	14 (6/8)
Highly probable	40 (16/24)	22.86	32 (12/20)

Table 2 Impact of allergy status on risk of *P. falciparum* clinical episodes. Shown are the *P* values and adjusted Odds Ratios with 95% confidence intervals calculated from the mixed model analyses. Values for the covariables Age (≥3.5 years of age compared to <3.5 years of age), Trimestrial incidence of *P. falciparum* clinical episodes and HbAS (beta-globin sickle cell trait; AS compared to AA) are those from the Asthma model analysis. For clarity significant co-variables are shown in bold.

	Age groups <3.5 years>	ORa	95% Confidence Intervals		*P* value
			Lower	Upper	
Atopy	**Both**	**1.65**	**1.20**	**2.26**	**2.0×10^{-3}**
	<3.5	1.38	0.92	2.08	0.124
	≥3.5	**2.02**	**1.39**	**2.93**	**2.1×10^{-4}**
Asthma	**Both**	**2.12**	**1.46**	**3.08**	**8.0×10^{-5}**
	<3.5	1.50	0.90	2.50	0.122
	≥3.5	**2.33**	**1.50**	**3.61**	**1.5×10^{-4}**
Atopic dermatitis	Both	1.05	0.65	1.70	0.842
	<3.5	0.84	0.49	1.46	0.539
	≥3.5	**3.15**	**1.56**	**6.33**	**1.3×10^{-3}**
Rhinoconjunctivitis	Both	0.96	0.65	1.41	0.818
	<3.5	1.05	0.64	1.72	0.853
	≥3.5	0.95	0.60	1.52	0.834
Age ≥3.5		**0.48**	**0.40**	**0.57**	**2.7×10^{-15}**
Trimestrial incidence		**1.01**	**1.00**	**1.01**	**1.8×10^{-6}**
HbAS		**0.24**	**0.12**	**0.47**	**3.7×10^{-5}**

Table 3 Impact of allergy status on the maximum *P. falciparum* parasite density during a clinical malaria episode. Shown are the back-transformed mean parasite densities per microlitre and standard errors (SEM) estimated from the GLMM analyses after taking into account the other co-variables. Significantly different effects are shown in bold for clarity.

Allergic condition	Age groups	Allergic status (No/Yes)	Mean parasite density	SEM	*P* value
Atopy	**Both**	**N**	**76.3**	13.8	
		Y	**131.0**	36.4	**0.0158**
	<3.5	N	114.3	23.7	
		Y	171.1	56.0	0.148
	≥3.5	**N**	**48.4**	9.8	
		Y	**114.8**	37.1	**$9.5x10^{-4}$**
Asthma	**Both**	**N**	**78.1**	14.4	
		Y	**148.5**	44.3	**$3.8\ x10^{-3}$**
	<3.5	N	114.8	24.3	
		Y	171.9	74.5	0.167
	≥3.5	**N**	**51.3**	9.7	
		Y	**105.3**	41.0	**$6.2\ x10^{-3}$**
Atopic dermatitis	Both	N	82.6	15.0	
		Y	93.9	38.9	0.605
	<3.5	N	122.6	25.5	
		Y	133.9	63.5	0.425
	≥3.5	**N**	**52.3**	11.0	
		Y	**135.4**	70.7	**0.014**
Rhinoconjunctivitis	Both	N	81.5	14.8	
		Y	111.4	39.0	0.570
	<3.5	N	118.8	25.1	
		Y	166.3	69.9	0.537
	≥3.5	N	54.6	11.3	
		Y	80.9	33.7	0.327

Figure 1 Annual incidence rate of clinical *P. falciparum* episodes per 100 children (bold line). In order to overcome the fluctuations of the annual incidence rate, we fit second (dotted line), third (dashed line) and fourth (solid line) degree polynomial trend lines to the data (bold line). The corresponding R-squared values are 0.70, 0.91 and 0·99 respectively indicating an accurate fit for third and fourth order polynomials. The inflexion on these two trend lines indicates the onset of acquisition of clinical immunity at approximately 3 to 4 years of age.

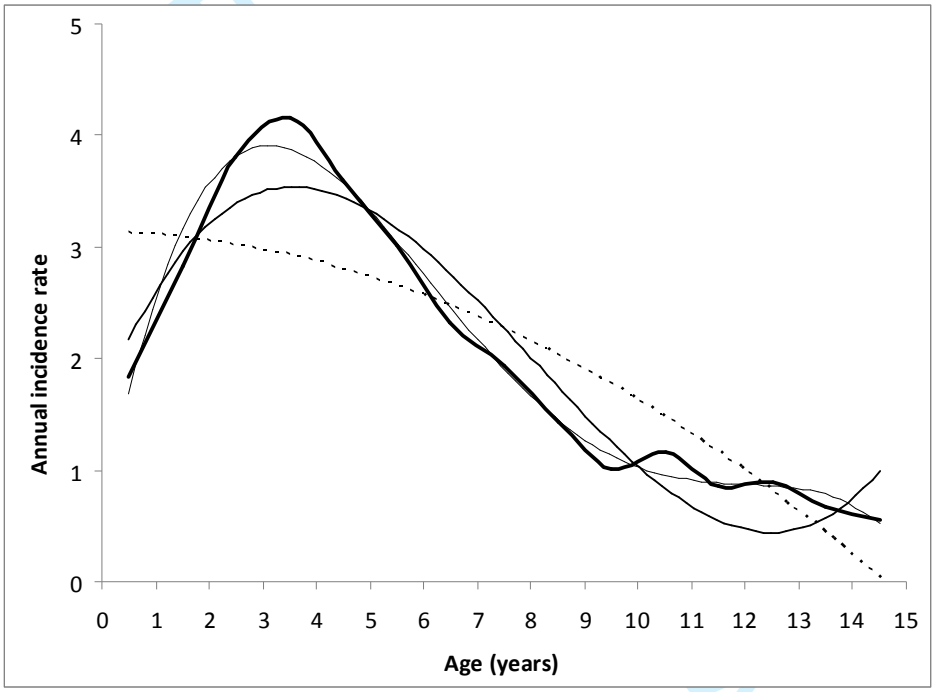
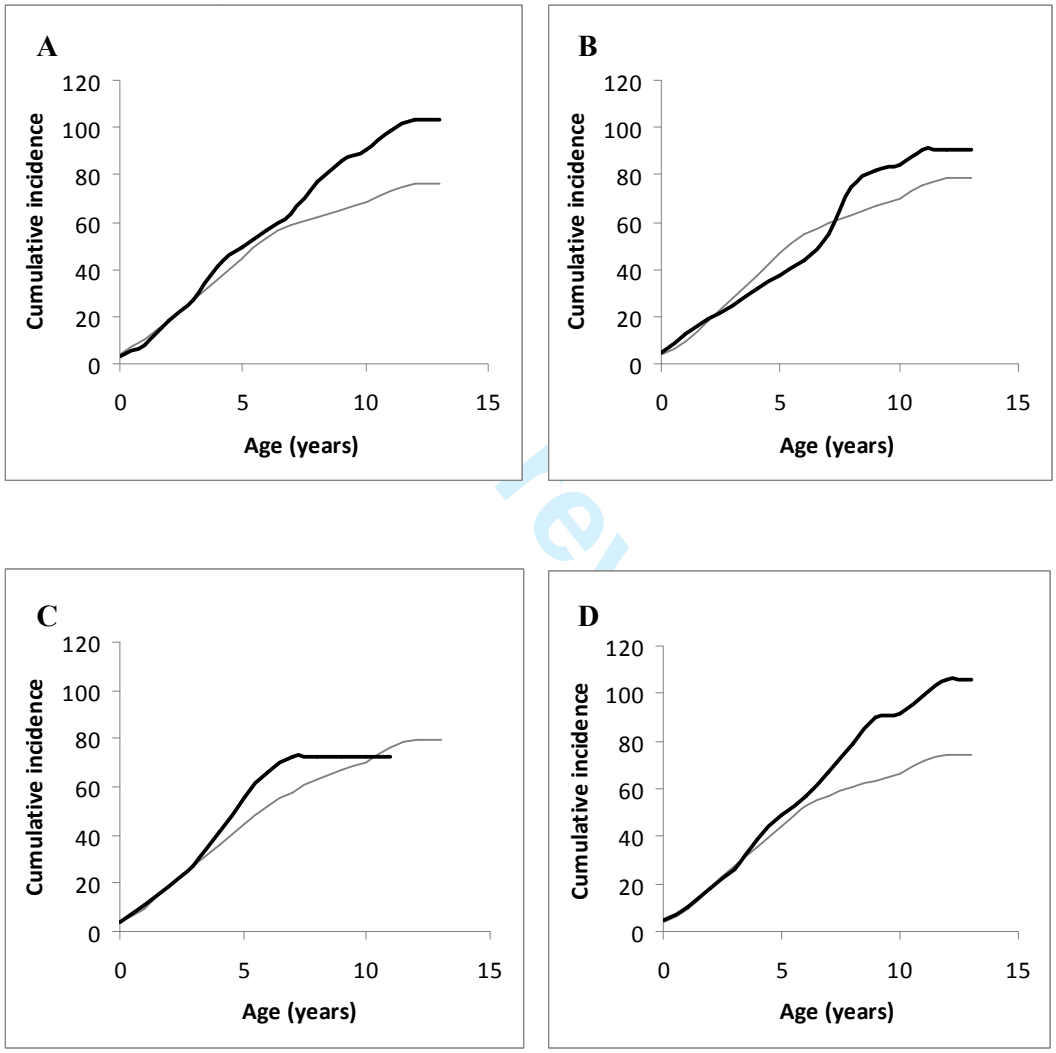


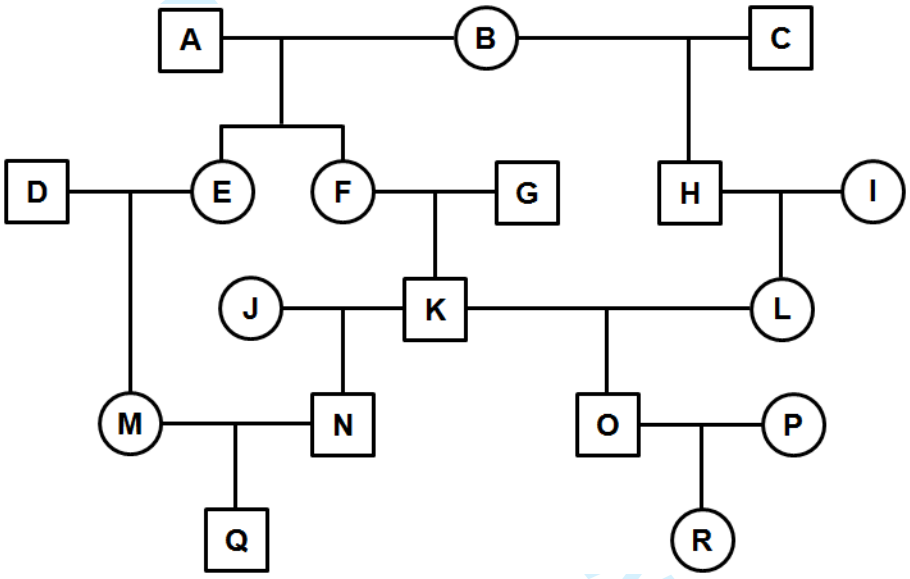
Figure 2 Mean cumulative number of *P. falciparum* clinical episodes with age for the (A) Asthma, (B) Rhinoconjunctivitis and (C) Atopic dermatitis classes and overall Atopy class (D) (bold lines) compared to individuals without symptoms of each respective allergy type (thin lines). In all cases moderate and severe classes are combined and compared to individuals without allergy symptoms. Note there are no children older than 11 years of age with Atopic dermatitis.



Pedigree-based genetic relatedness

The Genetic covariance between two individuals can be computed using the pedigree information. For individuals A and B, a given pair in a pedigree, the genetic covariance is computed as $r(A,B) = 2\times coancestry(A,B)$ where the *coancestry* between A and B is calculated referring to the method presented by Falconer and Mackay in 1996 (Falconer and Mackay 1996): $coancestry(A,B) = \Sigma_p(1/2)^{n(p)}\times(1 + I_{Common\ Ancestor})$ where p is the number of paths in the pedigree linking A and B, $n(p)$ the number of individuals (including A and B) for each path p and I_X is the *inbreeding* coefficient of X also equal to the *coancestry* between the two parents of X, I_X is set to 0 if X is a founder.

Illustration: Consider, as an example, the pedigree below containing 18 individuals named {A, B, …, R} for the calculation of genetic covariance's.



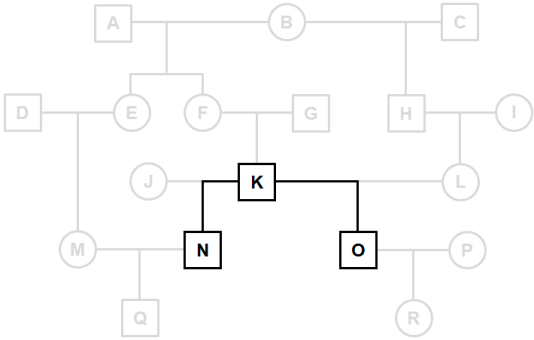
Pedigree structure.

The genetic relatedness between individuals N and O is equal to 0.266. This value is calculated as followed:
The number of paths linking N and O from the pedigree structure above is $p = 2$.
As illustrated below:

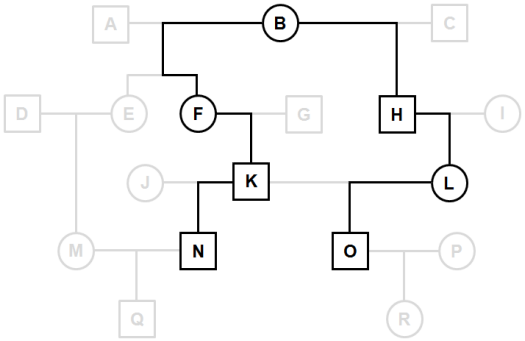
- **Path 1** contains $n(1) = 3$ individuals {N, K, O} with K as the common ancestor. Inbreeding coefficient of K, I_K , is the *coancestry* between the two parents of K (F and G) and is null because F and G are not genetically linked.
- **Path 2** contains $n(2) = 7$ individuals {N, K, F, B, H, L, O} with B as the common ancestor. Inbreeding coefficient of B, I_B , is null because B is a founder.

Therefore, genetic relatedness between individuals N and O is:

$$= 2\times(0.5^{n(1)}\times(1+I_K) + 0.5^{n(2)}\times(1+ I_B))$$
$$= 2\times(0.5^{3}\times(1+0) + 0.5^{7}\times(1+0)) = 0.266$$



Path 1 linking N and O.



Path 2 linking N and O.

Defining an equivalent model design where individual effects are independent using the genetic relatedness matrix:

Let us rename $Y^* = l(\mu)$. Y^* can be consider as a linearization of the phenotype through the link function l. The expected mean of Y^* and the variance of Y^* are:

(i) $E(Y^*) = E(X\beta + Z\gamma + \varepsilon)$
$= E(X\beta) + E(Z\gamma) + E(\varepsilon) = X \times E(\beta) + Z \times E(\gamma) + E(\varepsilon)$
$= X\beta$ (asymptomatically).

(ii) $Var(Y^*) = Var(X\beta + Z\gamma + \varepsilon)$
$= Var(Z\gamma + \varepsilon)$ (as $X\beta$ is the fixed part, thus has variance equal to 0)
$= Var(Z\gamma) + Var(\varepsilon)$ (as γ and ε are independent)
$= Z \times Var(\gamma) \times Z^T + Var(\varepsilon)$ (Z^T is the transpose of Z)
$= Z(A\sigma_g^2)Z^T + I\sigma_r^2$
$= ZAZ^T\sigma_g^2 + I\sigma_r^2$

If individuals were independent, i.e. $A = I_N$, variance of Y^* could be expressed as $ZZ^T\sigma_g^2 + I\sigma_r^2$. However, using linear algebra theory by the method "Cholesky decomposition of a matrix", we can show that there is an equivalent expression of the variance of Y^* corresponding to the modeling of data from independent individuals, having γ^* as an equivalent vector of random effects and Z^* an equivalent design matrix relating γ^* to Y^* so that:
$Var(Y^*) = Z^*(I\sigma_g^2)Z^{*T} + I\sigma_r^2$. $I\sigma_g^2$ is then the covariance matrix of the equivalent independent random individual effects γ^*.

Theorem: *Cholesky decomposition of a matrix*
If A is a symmetric positive-definite matrix, there is a triangular matrix L so that A can be written as $A = LL^T$. L can be seen as the "square root" of the matrix A.
Note that the genetic relatedness matrix A computed using the pedigree information (Falconer and Mackay 1996) is a positive-definite matrix, unless identical twins are in the pedigree in which case it would be positive semi-definite.

Equivalent model with independent random effects: We set $A = LL^T$ then:
$Var(Y^*) = Z(A\sigma_g^2)Z^T + I\sigma_r^2$
$= Z(LL^T\sigma_g^2)Z^T + I\sigma_r^2$

$= ZLL^{T}Z^{T}\sigma_g^2 + I\sigma_r^2$
$= (ZL)(ZL)^{T}\sigma_g^2 + I\sigma_r^2$
$= (Z^*)(Z^*)^{T}\sigma_g^2 + I\sigma_r^2$ (where we set $Z^* = ZL$)

Then, if we define $\gamma^* = L^{-1}\gamma$, we can rewrite the model as:

$Y^* = X\beta + Z^*\gamma^* + \varepsilon$ (because $Z\gamma = Z(LL^{-1})\gamma = (ZL)(L^{-1}\gamma) = Z^*\gamma^*$),

and the γ_i^* are independent, in other terms $Var(\gamma^*) = I\sigma_g^2$, as demonstrated below:

We assumed that $\gamma \sim N(0, A\sigma_g^2)$. Then $\gamma^* = L^{-1}\gamma$ is also distributed as a multivariate Normal with mean $E(\gamma^*) = L^{-1}E(\gamma) = L^{-1}\times 0 = 0$ and variance:

$Var(\gamma^*) = (L^{-1})\times Var(\gamma)\times(L^{-1})^{T}$
$= (L^{-1})\times A\sigma_g^2\times(L^{-1})^{T} = (L^{-1})LL^{T}(L^{-1})^{T}\sigma_g^2$
$= (L^{-1}L)(L^{-1}L)^{T}\sigma_g^2$
$= I\sigma_g^2$

The random effects are now independent and then the classical mixed model assuming independence between levels (here individuals) is applied, and the estimate of fixed effects obtained are fine, i.e. corrected for genetic relationships.

References

Falconer DS, Mackay TFC (1996) Introduction to Quantitative Genetics. 4th Edn. London: Longman.

Supplementary Tables

Table S1 Number of person-trimesters contributed by number of children by age class and the number who had severe/moderate allergy symptoms, for whom malaria data were also available. AS – Asthma, AD – Atopic dermatitis, RC – Rhinoconjunctivitis. Shown also are the numbers of these individuals suffering from two or all three allergy conditions.

Age group	N° person-trimesters	N° people	AS	AD	RC	AS+AD	AS+RC	AD+RC	AS+AD+RC
]1	7	6	1	2	2	0	1	0	0
]2	21	9	0	1	3	0	0	0	0
]3	48	11	1	1	2	0	0	1	0
]4	119	12	1	2	3	0	0	1	0
]5	102	11	3	4	3	2	1	2	1
]6	125	11	1	1	0	0	0	0	0
]7	303	11	1	2	1	1	0	0	0
]8	340	12	1	1	1	1	0	0	0
]9	362	10	2	0	1	0	1	0	0
]10	610	17	1	0	3	0	0	0	0
]11	77	4	2	1	0	0	0	0	0
]12	484	16	3	0	3	0	1	0	0
]13	390	10	1	0	0	0	0	0	0
]14	105	3	0	0	1	0	0	0	0
Total	3093	143	18	15	23	4	4	4	1

Table S2 Summary of total number of person-trimesters with non-malaria and symptomatic *P. falciparum* clinical presentations and total number of non-malaria episodes according to age class. Given are the number of people contributing to each type of presentation.

	Age group (years)	
	<3·5	**≥3·5**
Total person-trimesters	1283	1810
People	126	113
Total *P. falciparum* symptomatic trimesters	963	1102
People	114	108
Total non-malaria episodes	754	1114
People	123	109

Table S3 Effect of changing age threshold on impact of allergy on the risk of clinical malaria and concomitant parasite density. Given are Odds Ratio with 95% confidence intervals, for clinical malaria episodes and the beta coefficient and standard error for parasite density. Corresponding P values are also given. Values are from the nested GLMM analyses.

A. Malaria episodes							**B. Parasite density**				
Age cut-off (years)	OR	95% CI	P value	OR	95% CI	P value	Age cut-off	beta coeff (se)	P value	beta coeff (se)	P value
Atopy		above threshold			below threshold		Atopy	above threshold		below threshold	
1.5	1.80	1.25-2.59	1.7x10-3	1.57	0.85-2.89	0.15	1.5	0.70 (0.27)	9.2x10-3	0.54 (0.35)	0.12
2.5	2.00	1.39-2.88	2.0x10-4	1.23	0.76-1.99	0.40	2.5	0.79 (0.26)	2.6x10-3	0.35 (0.29)	0.23
3.5	2.02	1.39-2.93	2.1x10-4	1.38	0.92-2.08	0.12	3.5	0.85 (0.26)	9.5x10-4	0.37 (0.26)	0.15
4.5	2.10	1.42-3.10	1.6x10-4	1.41	0.98-2.04	0.063	4.5	0.87 (0.25)	6.9x10-4	0.40 (0.23)	0.09
5.5	1.64	1.07-2.52	0.02	1.67	1.17-2.37	0.004	5.5	0.73 (0.27)	7.4x10-3	0.48 (0.22)	3.4x10-3
Asthma							Asthma				
1.5	1.98	1.29-3.03	1.8x10-3	1.46	0.69-3.19	0.34	1.5	0.66 (0.31)	0.03	0.30 (0.44)	0.48
2.5	2.30	1.49-3.55	1.6x10-4	1.15	0.63-2.09	0.65	2.5	0.78 (0.30)	0.01	0.26 (0.36)	0.48
3.5	2.33	1.50-3.61	1.5x10-4	1.50	0.90-2.50	0.12	3.5	0.82 (0.30)	6.2x10-3	0.43 (0.31)	0.17
4.5	2.30	1.48-3.59	2.4x10-4	1.76	1.11-2.80	0.017	4.5	0.81 (0.29)	5.8x10-3	0.56 (0.28)	0.049
5.5	1.98	1.22-3.22	0.006	2.06	1.33-3.18	0.0011	5.5	0.72 (0.31)	0.02	0.62 (0.27)	0.02
Atopic Dermatitis							Atopic Dermatitis				
1.5	2.05	1.18-3.56	0.01	0.91	0.42-1.97	0.80	1.5	0.80 (0.37)	0.03	0.72 (0.46)	0.12
2.5	2.49	1.36-4.57	3.1x10-3	0.82	0.44-1.53	0.53	2.5	0.77 (0.38)	0.044	0.52 (0.39)	0.19
3.5	3.15	1.56-6.33	1.3x10-3	0.84	0.49-1.46	0.54	3.5	0.99 (0.40)	0.014	0.28 (0.35)	0.42
4.5	3.79	1.61-8.92	2.3x10-3	0.94	0.57-1.57	0.82	4.5	0.98 (0.47)	0.036	0.29 (0.32)	0.37
5.5	1.33	0.47-3.77	0.59	1.19	0.73-1.96	0.49	5.5	0.26 (0.61)	0.67	0.38 (0.31)	0.22
Rhinoconjunctivitis							Rhinoconjunctivitis				
1.5	1.04	0.66-1.62	0.88	1.01	0.51-2.01	0.98	1.5	0.36 (0.32)	0.27	0.18 (0.41)	0.66
2.5	1.01	0.64-1.61	0.96	0.96	0.55-1.68	0.89	2.5	0.28 (0.33)	0.40	0.25 (0.35)	0.48
3.5	0.95	0.60-1.52	0.83	1.05	0.64-1.72	0.85	3.5	0.31 (0.32)	0.33	0.19 (0.31)	0.54
4.5	0.87	0.54-1.42	0.59	1.06	0.68-1.66	0.79	4.5	0.20 (0.32)	0.53	0.22 (0.28)	0.44
5.5	0.81	0.48-1.36	0.43	1.07	0.70-1.64	0.74	5.5	0.10 (0.33)	0.75	0.23 (0.27)	0.39

Table S4 Frequency of non-malaria episodes (number of days of presence divided by number of non-malaria episodes) according to allergic status and age group. The *P* value is that from the GLMM analyses of the effect of allergic status by age group on the number of non-malaria episodes per person-trimester.

Allergic condition	Allergic status (No/Yes)	Age group (years) <3·5	Age group (years) >3·5	*P* value
Atopy	N	78·2	85·9	0·105
	Y	87·2	102·6	
Asthma	N	79·6	87·3	0·319
	Y	82·5	100·2	
Atopic dermatitis	N	80·9	88·2	0·323
	Y	73·4	101·9	
Rhinoconjunctivitis	N	77·9	88·3	0·167
	Y	94·9	91·8	

Figure S1. Incidence of clinical cases per 100 person-trimesters in children under 15 years of age.

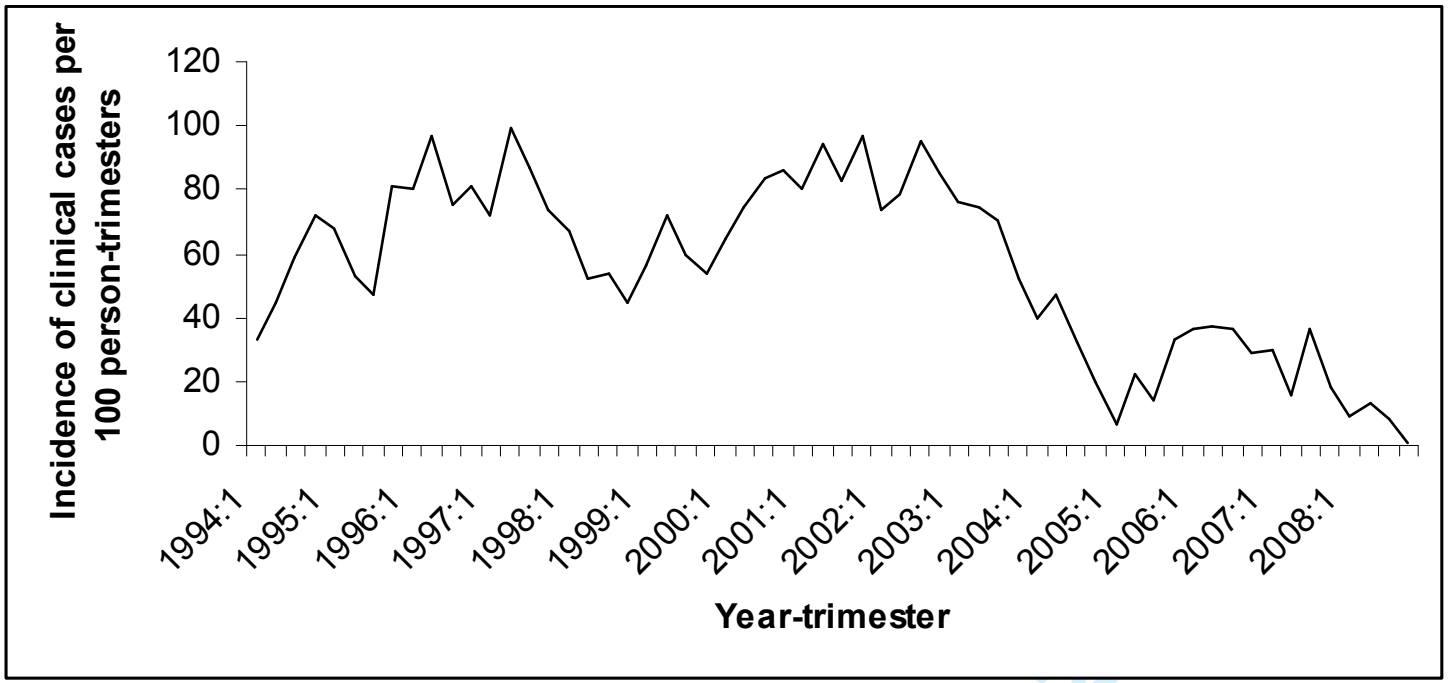


Figure S2. Cumulative incidence of clinical cases according to allergy class predicted by the statistical model.

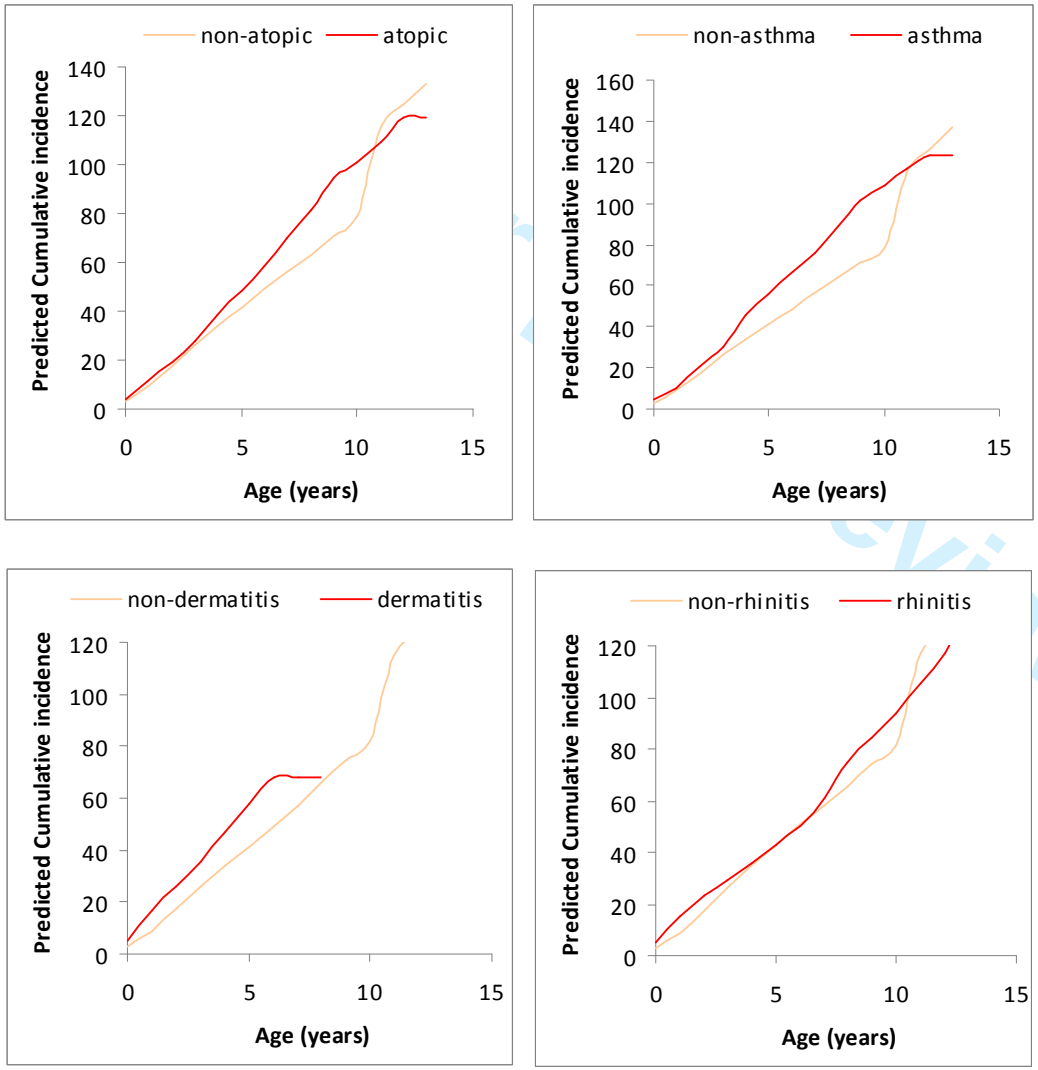
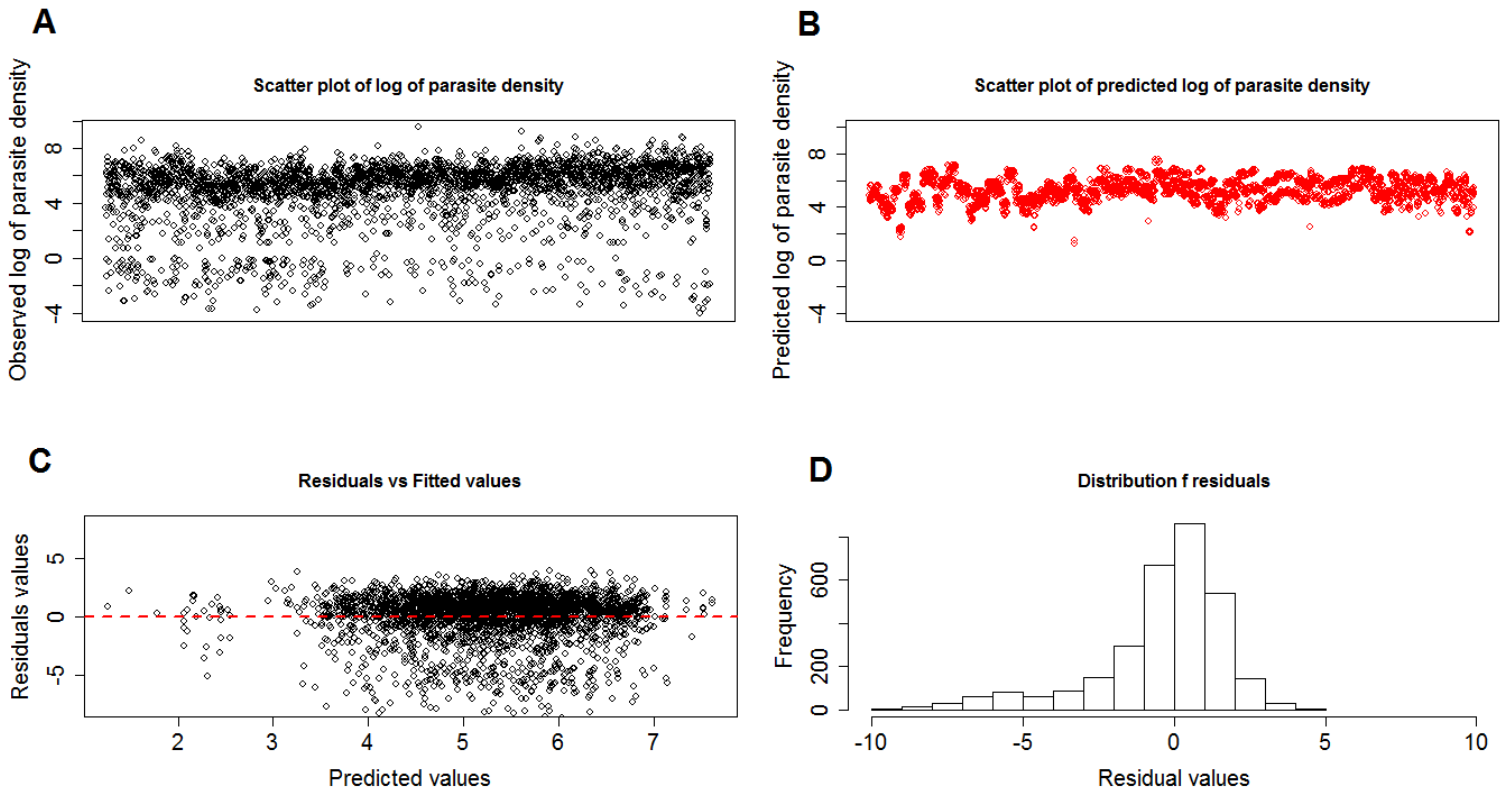


Figure S3. Graphical control model for parasite density

These figures provide a graphical checking of model goodness of fit. Figure A is the scatter plot of the natural logarithm of the observed parasite density and is compared to Figure B, which is the scatter plot of the natural logarithm of the predicted parasite density by the model; on both figures A and B the y-axes give the values for the log of the parasite density. Figure C shows the distribution of the residuals with the predicted values and Figure D is the histogram of the residuals; both figures C and D show the residuals normally distributed around zero.



Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium falciparum* malaria

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Article summary

Article focus

- Genetic studies suggest a link between susceptibility to allergy and malaria in Africa
- We hypothesize that atopy increases susceptibility to malaria

Key messages

- Results demonstrate an association between asthma, atopic dermatitis and susceptibility to clinical *P. falciparum* episodes.
- Genetic pre-disposition to asthma or atopic dermatitis impairs the acquisition of clinical immunity to malaria.
- Administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Strengths and limitations

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association.

Abstract

Objectives: To assess the impact of atopy and allergy on the risk of clinical malaria.

Design: A clinical and immunological allergy cross-sectional survey in a birth cohort of 175 children from 1 month to 14 years of age followed for up to 15 years in a longitudinal open cohort study of malaria in Senegal. Malaria incidence data were available for 143 of these children (aged 4 months to 14 years of age) for up to 15 years. Mixed model regression analysis was used to determine the impact of allergy status on malaria incidence, adjusting for age, sickle cell trait and force of infection.

Main outcome measures: Asthma, allergic rhino-conjunctivitis and atopic dermatitis status, the number of clinical *Plasmodium falciparum* malaria episodes since birth and associated parasite density.

Results: Twelve percent of the children were classified as asthmatic and ten percent as having atopic dermatitis. These groups had respectively a two-fold (OR 2.12 95% confidence intervals 1.46% to 3.08%; P= 8 x10^{-5}) and three-fold (OR 3.15, 1.56% to 6.33%; P= 1.3 x10^{-3}) increase in the risk of clinical *P. falciparum* malaria once older than the age of peak incidence of clinical malaria (3 to 4.5 years of age). They also presented with higher *P. falciparum* parasite densities (Asthma: mean 105.3 parasites/µL ±SE 41.0 *vs*. 51.3 ±9.7; P= 6.2 x10^{-3}; Atopic dermatitis: 135.4 ±70.7 *vs*. 52.3 ±11.0; P=0.014). There was no effect of allergy on the number of non-malaria clinical presentations. Individuals with allergic rhinoconjunctivitis did not have an increased risk of clinical malaria nor any difference in parasite densities.

Conclusion: These results demonstrate that asthma and atopic dermatitis delay the development of clinical immunity to *P. falciparum*. Despite the encouraging decrease in malaria incidence rates in Africa, a significant concern is the extent to which the increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines, administration to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Introduction

The World Allergy Organization estimates that 40% of the world's population is concerned by allergic diseases.[1] In developing countries where *Plasmodium falciparum* malaria is endemic, prevalence of allergy is significantly lower, but is on the increase.[2] Orientation of the immune response towards a Th1 profile is crucial for immunity to intracellular pathogens,[3] whereas orientation towards a Th2 profile drives immunity to extracellular pathogens and antigens resulting in class switching giving rise to IgE-producing B cells.[4] An important role of the Th1/Th2 balance in the development of clinical malaria following infection by *P. falciparum* has been suggested by numerous studies.[5-7] It has been suggested that the Th2 bias induced by *P. falciparum* may exacerbate allergy.[8] Likewise, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*. However, the interplay between infectious agents and allergy is unclear. On the one hand, for example, severe respiratory syncytial virus infection in infants increased the risk of allergic rhinoconjunctivitis and allergic asthma.[9,10] On the other hand, measles,[11] hepatitis A[12] and tuberculosis[13] seemingly reduce atopy. Although, an atopic condition can increase incidence of disease, such as the case for the skin commensal *Staphylococcus aureus* in patients with atopic dermatitis,[14] an atopic tendency *per se* does not generally lead to increased illness from infectious agents.

~~*Plasmodium falciparum* specific IgE is elevated in malaria patients and has been proposed to play a pathogenic role in severe malaria.[3] T helper type 2 (Th2) cell bias induced by *P. falciparum* may exacerbate allergy.~~[4] Genome wide studies have identified chromosomal regions linked to clinical malaria, all of which overlap with those previously identified to be involved in atopic dermatitis, asthma, atopy and IgE levels[5-7],[15-17] suggesting that common mechanisms may be involved in both pathologies.[18~~8~~] Chromosomal region 5q31 that has been repeatedly shown to be associated with control of parasite density and contains a cluster of cytokines, among which IL12B has been previously associated with psoriasis.[19] The other regions, 13q13-q22, 5p15-p13 and 12q21-q23, contain genes involved in innate immunity, notably the interleukin 7 receptor, and several involved in tumour necrosis factor synthesis [C1q and tumour necrosis factor related protein 3 (C1QTNF3)] and a gene involved in the complement system (C9).[18]

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Several additional lines of evidence support the concept that susceptibility to malaria and atopy may be related to similar immunological defects. In Ethiopia, a history of malaria was associated with atopy.[9,20] A mouse model for human atopic disease was found to be very susceptible to murine malaria and a major locus for atopic disease mapped close to the region controlling parasite density.[21,10] This region contains several candidate genes that have effects on T-cell function.[21]

Moreover, a direct effect of histamine in the malaria pathogenesis has been found using genetic and pharmacological approaches[11,22] and increased levels of histamine are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[12,21,13,24]

To test the hypothesis that allergy impacts upon clinical *P. falciparum* malaria, we performed a clinical allergy cross-sectional study in the family-based longitudinal cohort from Senegal previously used for the genome linkage study[8,18] and analysed the impact of asthma, atopic dermatitis, allergic rhinoconjunctivitis on the incidence of clinical *P. falciparum* episodes and the maximum parasite density during each episode.

Methods

Population and outcome data

The malaria research program conducted in Dielmo village in Senegal has been ongoing since 1990 as described elsewhere.[25,54] In brief, between 1990 and 2008, a longitudinal study involving the inhabitants of the village of Dielmo, Senegal, was carried out to identify all episodes of fever. The study design included daily medical surveillance with systematic blood testing of individuals with fever and examination of 200 oil-immersion fields on a thick blood film for malaria parasites (about 0.5 µL of blood). Each individual was given a unique identification code and details of family ties, occupation, and precise place of residence were recorded on detailed maps of each household with the location of each bedroom. All households were visited daily, absenteeism recorded, and the presence of fever or other symptoms assessed. We systematically recorded body temperature at home three times a week (every second day) in children younger than 5 years, and in older children and adults in cases of suspected fever or fever-related symptoms. In cases of fever or other symptoms,

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blood testing was done at the dispensary by finger prick, and we provided detailed medical examination and specific treatment. Parasitologically confirmed clinical malaria episodes were treated according to national guidelines. From 1990 to 2008, four different drug regimens were implemented: Quinine from 1990 to 1994, Chloroquine from 1995 to 2003, Fansidar (sulfadoxine-pyrimethamine) from 2004 to mid-2006 and Artemisinin-based combination therapy (ACT; Amodiaquine- sulfadoxine-pyrimethamine) from mid-2006 to 2008.

Parasite positivity was established as follows. Thick blood films were prepared and stained by 3% Giemsa stain. Blood films were examined under an oil immersion objective at x1000 magnification by the trained laboratory technicians and 200 thick film fields were examined to count the number of asexual and gametocyte parasite stages. Asexual parasite densities (per µL) were calculated by establishing the ratio of parasites to white blood cells and then multiplying the parasite count by 8,000, the average white blood cell count per µL of blood.

Malaria transmission in Dielmo is intense and perennial. We conducted a cross-sectional survey to estimate the prevalence of symptoms related to allergic diseases among 175 children aged from 1 month to 14 years old ~~below the age of 15 years~~ who were born during the malaria research program.

Both the longitudinal and cross-sectional surveys were approved by the Ministry of Health of Senegal. Informed consent of the volunteers is renewed every year. More specifically for the cross-sectional survey, after informing about the procedures and the purpose of the study, written informed consent was obtained from parents or guardians of children either by signature or by thumbprint on a voluntary consent form written in both French and Wolof, the main local language. Consent was obtained in the presence of the school director, an independent witness.

The family structure (pedigree) was available after a demographic census performed for every volunteer at his adhesion in the project. A verbal interview of mothers or key representatives of the household was used to obtain information on genetic relationships between studied individuals, their children, their parents, and to identify genetic links among the population. The total pedigree comprised 828 individuals, including absent or dead relatives, composed of ten independent families that can be sub-divided into 206 nuclear families (father – mother couples with at least one child) with an average of 3.6

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children each. Genetically related nuclear families occur because of multiple marriages and marriages among related individuals. Previous typing with microsatellites has enabled the construction of a pedigree based on Identity-by-Descent using MERLIN.[18,26] The mean coefficient of inbreeding is 0.0008. Newborns since this original genetic analysis were added to the family of the parents in question. The 143 children, with both allergy and malaria data, belonged to 61 nuclear families and comprised 30 singletons, 102 siblings and 11 half-sibs (yielding 55 half-sib pairs). The mean genetic relatedness (by pedigree) of the 143 children is 0.0114 (range: 0.0013 to 0.022).

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P. falciparum *clinical episodes*

P. falciparum malaria clinical episode phenotypes analysed were: (i) clinical *P. falciparum* infections treated with anti-malarial therapy and (ii) the highest parasite density during the *P. falciparum* clinical episode. A clinical *P. falciparum* episode was defined as a clinical presentation with fever (axillary temperature ≥ 37.5°C) and/or other clinical signs suggestive of malaria associated with a thick blood smear positive for *P. falciparum* and that was treated with anti-malarial therapy~~. The parasite density was measured from 200 microscope fields on a thick blood smear (0.25 µL blood)~~. Repeated clinical malaria presentations within 15 consecutive days were not considered to be independent and were excluded from the analyses, unless there was a negative thick blood smear between two clinical presentations. We also excluded observations in any trimester for which the individual was not present for at least one third of the time.

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We calculated the quarterly incidence rate of clinical *P. falciparum* episodes in children below the age of 15 years as the ratio of the total number of clinical *P. falciparum* episodes during the trimester divided by the total number of person-trimesters surveyed. Incidence rate is expressed as cases per 100 person-trimesters (see Supplementary Figure S1). This rate was used in the analysis to approximate the force of infection (exposure level) within the targeted population at the time of a given clinical *P. falciparum* episode.

The total number of clinical presentations per trimester that were not attributable to *P. falciparum* was tabulated. Repeated non-malaria presentations within seven consecutive days were not considered to be independent and were excluded.

Allergic diseases and atopic status

The International Study of Asthma and Allergies in Childhood (ISAAC) diagnostic criteria have been shown to be reproducible, adequate and able to discriminate children with allergic diseases in different areas of the world.[2] The standardized ISAAC questionnaire originally written in English was translated into French in compliance with ISAAC guidelines[15~~27~~], adapting it to the usual local customs following advice from local clinicians and paediatric allergologists (Acknowledgements and Technical Appendix). The adequacy and reliability of the translated questionnaire had been previously confirmed by a pilot study on 30 randomly selected children in the same community. The questionnaire was completed by specially trained health workers during an oral interview conducted in Wolof with children and their mothers or guardians.

To assess the prevalence of allergic diseases in children, we used the positive and negative predictive values of the ISAAC questionnaire diagnosis criteria developed for subtropical countries.[16~~28~~] Each question was scored according to the medical diagnosis of paediatricians and paediatric allergologists. Positive or negative answers were thus graded on the basis of symptom sensitivity, specificity, frequency, location or early onset. For each allergic disease, three categories of symptom severity, *severe*, *moderate*, and *none*, were defined as follows:

Asthma – severe symptoms if the child had "wheezing or whistling in the chest before the age of two years" and "more than three times" *or* severe enough to "limit his/her speech"; *moderate* symptoms if the child had "wheezing or whistling in the chest before the age of two years" and "in the past 12 months"; and *none* otherwise.

Allergic rhinoconjunctivitis – severe symptoms if the child had "sneezing, runny or stuffy nose in the past 12 months" and "more than five times a year", and "itchy, watery eyes or tropical endemic limboconjunctivitis (TELC) in the past 12 months"; *moderate* symptoms if the child had "sneezing, runny or stuffy nose in the past 12 months", and "itchy, watery eyes or TELC in the past 12 months"; and *none* otherwise.

Atopic dermatitis – severe symptoms if the child had "scaly or exudating, crusted and pruritic patches in the past 12 months" and "affecting any of the following characteristic areas: face, around the ears or eyes, folds of armpits or elbows or groin, behind the knees, under the buttocks", and "onset of symptoms before the age of two years"; *moderate* symptoms if the

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child had “scaly or exudating, crusted and pruritic patches in the past 12 months” and “affecting any of characteristic areas (see above)”, and “onset of symptoms before the age of four years”; and *none* otherwise.

The inter-relationships between variables reflecting the severity of symptoms of the three allergic diseases were used to identify children at high risk of atopy. The *high probability* group was defined by the prevalence of at least one of any *severe* symptoms or two of any *moderate* symptoms. The *probable* group was defined as those with *moderate* symptoms from one of the three allergic diseases and remaining children were classified in the *unlikely* group.

Helminths

Helminthic infections are common in this region and are known to modify the clinical course and outcome of both allergic diseases and malaria.[29,30] We therefore carried out a~~A~~ helminth survey ~~was carried out~~ for 91 individuals present during the cross-sectional survey. Diagnosis was performed by stool examination by microscope and by the Kato technique to search for the presence of *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*), whipworm (*Trichuris trichiuria*), *Schistosoma mansoni*, and *Strongyloides stercoralis*. Examination for pinworms (*Enterobius vermicularis*) was performed by the anal scotch-test. An anti-helminthic treatment was proposed for all infested individuals.

Immunoglobulin E titres

Specific IgE titres were measured by ELISA as previously described.[31~~17~~] A panel of allergens of potential pertinence to the three classes of allergy was used: (i) Salivary gland extracts (SGE) of two mosquito species present in the study cohorts, *Aedes aegypti* and *Anopheles gambiae sensu stricto*, and (ii) *P. falciparum* parasite extract were prepared as previously described[31~~17~~]; (iii) House dust mite spp. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*; (iv) a mix of pollen allergens from five ubiquitous graminae spp. [Cock’s-foot (*Dactylis glomerata*), Timothy grass (*Phleum pratense*), Sweet Vernal grass (*Anthoxanthum odoratum*), Perennial ryegrass (*Lolium perenne*), Kentucky Bluegrass (*Poa pratensis*)] (all from Stallergenes, France).

Statistical analysis

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Statistical analyses were performed using R version 2.12.0 (The R Foundation for Statistical Computing, Vienna, Austria). To address the effect of allergic status on the risk of clinical *P. falciparum* episodes, we performed Generalized Linear Mixed Models (GLMM) extended to pedigree data using the *pedigreemm* package for R to account for the non-independence of individuals because of family relationships, shared house and for repeated measures from the same individual (Technical Appendix). Correlated individual effects due to familial relationships were taken into account by using the pedigree-based genetic relatedness matrix that contains the genetic covariance among all pairs of individuals in the study cohort and is calculated using the pedigree information.[~~18~~32] Shared house and repeated measures from the same individual were modelled as random effects. All random effects were assumed to be normally distributed, and conditional on these random effects, the dependent variable had: (i) a Binomial distribution when the studied phenotype was the occurrence of a clinical *P. falciparum* episode treated with anti-malarial therapy during a trimester, (ii) a Gaussian distribution when the studied phenotype was the logarithm of the maximum parasite density during a given clinical *P. falciparum* episode, and (iii) a Poisson distribution when the studied phenotype was the number of non-malaria episodes per trimester. The effects of allergy disease classes on these dependent variables were modelled as fixed effects. Allergy classes were reduced to two levels, *Severe* or *moderate vs*. *none* for analyses of asthma, atopic dermatitis and allergic rhinoconjunctivitis and *high probability vs*. *probable* and *unlikely* for atopic tendency. Co-variables included sickle cell trait[~~17~~31], gender, number of days present on site during the trimester, trimestrial incidence of *P. falciparum* and age. Age was initially analysed as a continuous covariate. To assess the age-specific effect of allergy, age was categorised into two levels (<3.5 years of age and ≥3.5 years of age, based on the age of peak clinical incidence) and allergy class was nested within age class. The age threshold was varied from 1.5 years to 5.5 years of age and the data re-analysed to assess at which age there was the strongest effect. The association of allergy classes with IgE levels was analysed by box-cox transforming the data and fitting a GLMM with ~~Poisson~~ a normal distribution.

Results

Of the 205 eligible children aged under 15 years involved in the family-based longitudinal study, 175 (85.4 %) participated in the cross-sectional survey to assess the prevalence of related symptoms of allergic diseases. All eligible children present at the time of the survey were included; no explicit refusal to participate was recorded. The study cohort was aged from 1 month to 14 years 11 months. The sex-ratio (male/female) was 0.94.

From 1994 until 2008, 143 of the children participating in the cross-sectional survey were present for at least 31 days in any trimester during the study period generating a total of 3,093 person-trimesters of presence (Supplementary Table S1). There were 2,065 treated *P. falciparum* clinical episodes (median 11, range 0-47)(Supplementary Table S2). The age peak of incidence of *P. falciparum* episodes occurred at 3 to 4~~.5~~ years of age (Figure 1). There were 1,868 non-malaria episodes (median 12, range 0-37) (~~Supplementary~~ Table S2). These non-malaria clinical presentations were associated with headache (38 %), chills (32 %), cough (13 %), vomiting (11 %) and diarrhoea (6 %).

The prevalence of moderate or severe asthma symptoms was respectively 2.3 % and 10.3 % (Table 1). The prevalence of moderate or severe allergic rhinoconjunctivitis symptoms was respectively 6.3 % and 10.3 %. The prevalence of moderate or severe atopic dermatitis symptoms was respectively 6.3 % and 2.9 %. On the basis of symptom severity, an atopic tendency was estimated to be unlikely for 68.0 %, probable for 9.1 % and highly probable for 22.9 % of the 175 children. The frequency of each allergy class in children for whom malaria data were available is shown in Table S1.

The risk of treated clinical *P. falciparum* infections was higher for children with high probability of atopy (OR 1.65, 95% confidence intervals 1.20 to 2.26; P=0.002) (Table 2), after adjusting for age, sickle cell trait and the exposure level. Gender was not found to be significant. Analysing the impact of atopy in children younger and older than the peak age of clinical incidence (3 to 4~~.5~~ years old), revealed that atopy ~~doubled~~ increased the risk of *P. falciparum* episodes in children at an age greater than 3.5 years (OR 2.02, 1.39 to 2.93; $P=2x10^{-4}$), but not in children of age prior to the peak clinical incidence (OR 1.38, 0.92 to 2.08; P=0.124) (Table 2). This increased risk resulted in an ever increasing cumulative number of *P. falciparum* episodes with age beyond that of peak clinical incidence (Figure 2. See supplementary Figure S2 for model predictions for comparison).

Analysis by allergy category revealed that asthma (severe or moderate) ~~doubled~~ increases the risk of *P. falciparum* episodes (OR 2.12, 1.46 to 3.08; P= 8 x10^{-5}) and this again only in children of age greater than 3.5 years old (OR 2.33, 1.50 to 3.61; P= 1.5 x10^{-4}). Atopic dermatitis ~~tripled~~ increased the risk of clinical malaria in children older (OR 3.15, 1.56 to 6.33; P= 1.3 x10^{-3}) but not younger than 3.5 years of age (Table 2). Allergic rhinoconjunctivitis was not associated with increased risk of clinical malaria at any age (Table 2). The impact of atopy, asthma and atopic dermatitis can be clearly seen in the ever-increasing number of cumulative *P. falciparum* episodes beyond the age of the onset of clinical immunity in the population, 3.5 years of age (Figure 2). There is no difference in the number of clinical malaria episodes prior to this age in individuals with or without an allergic condition. Analysis using different age thresholds (from 1.5 to 5.5 years of age) revealed similar OR for thresholds of 2.5, 3.5 and 4.5 years of age. The maximum OR for increased malaria occurred in children older than 4.5 years of age and with atopy or atopic dermatitis, whereas for the asthma group it occurred in children after 3.5 years of age (Supplementary Table S3). ~~There is no difference in the number of clinical malaria episodes prior to this age in individuals with or without an allergic condition.~~

There was no impact of any allergic disease on the number of non-malaria episodes by trimester (Supplementary Table S~~4~~3).

The impact of atopy, asthma and atopic dermatitis on the maximum *P. falciparum* parasite density during a given clinical malaria episode mirrored that of the risk of *P. falciparum* episodes. Parasite density was significantly higher for children with allergic disease older than 3.5 years of age (Table 3 and supplementary Figure S3 for residuals of the fitted model). Allergic rhinoconjunctivitis had no impact on the parasite density (Table 3). Analysis using different age thresholds yielded the same pattern as seen with the number of clinical episodes (Table S3).

Individuals with moderate or severe symptoms of atopic dermatitis had significantly higher specific IgE titres against *Ae. aegypti* (P=0.004) and *An. gambiae* SGE (P<0.001). There were no detectable specific anti-*P. falciparum* IgE. Individuals with moderate or severe symptoms of allergic rhinoconjunctivitis did not have significantly higher IgE titres against the tested graminae (P=0.28), although titres decreased with age (P=0.035). There was also no effect of

asthma on IgE titres against the house dust mite spp. tested (*D. farinae* P=0.60 & *D. pteronyssinus* P=0.27).

Only five individuals were infested with helminths (two *Ancylostoma*, one *Strongyloides*, one *Trichuris* and one *Enterobius*).

Discussion

Principal findings

Establishing the allergic status of children up to the age of 15 years old followed for malaria since birth, revealed an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

Strengths and weaknesses of the study

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association. In addition, although allergy diagnosis for children under 2 years of age is not considered reliable, there were only 15 individuals under 2 at the time of the allergy study of the 143 for whom malaria and allergy data were available.

Meaning of the study

Under intense malaria transmission, after repeated exposure to the parasite, children develop a clinical immunity[~~19~~33], whereby they tolerate elevated parasite densities without showing clinical symptoms. In this cohort, the population mean onset of clinical immunity occurred at 3 to 4~~.5~~ years of age. Although clinical immunity is accompanied by a reduction in parasite density, effective anti-parasite immunity develops much more slowly[~~20~~34] with individuals achieving a state of premunition, whereby they maintain low-grade parasite densities in an asymptomatic state.[~~21~~35] We show here that children with clinically defined

asthma or atopic dermatitis had a two to three-fold increase in the risk of presenting with *P. falciparum* malaria episodes requiring treatment once passing the age of peak clinical incidence. They also had higher parasite density during clinical episodes, suggesting a reduced ability to control parasite replication. The observed increase in clinical incidence of malaria in patients with asthma or atopic dermatitis is not likely to be the result of increased frailty of such individuals; these individuals did not come more frequently to the clinic with non-malaria symptoms. Our previous genome linkage study identifying chromosomal regions[8,18] associated with malaria that overlap with those previously shown to be linked to asthma/atopy suggests that there may be a shared genetic basis to these pathologies rather than any causative effect of one on the other. This is consistent with the increased susceptibility to malaria of mouse atopic models.[10,21]

Comparison with other studies

A previous study found that a history of malaria (yes/no) increased risk of atopic dermatitis in 306 cases compared to 426 controls as characterized using the ISAAC questionnaire[9,20]. The only other epidemiological study that has previously examined the link between malaria and atopy[22,36] also interpreted the result from the perspective of the impact of malaria on atopy. They examined the re-infection rate with *P. falciparum* over a 5-year period in 91 children that were subsequently classified as atopic or not using skin prick tests (SPT) with house dust mite antigen. Their conclusion was that, as with measles[23,11] and tuberculosis[24,13], malaria infection reduces atopy. However, the study lacked previous infection data since birth of the participating individuals and focussed on atopy as determined by SPT against a single allergen. The case-control study of atopic dermatitis risk factors cited above found no overall association between allergen skin sensitization and atopic dermatitis. We also found no evidence of increased IgE titres against house dust mites in the asthmatic or atopic dermatitis groups or against grass pollen in individuals with allergic rhinoconjunctivitis. Such differences likely reflect the different IgE reactivity profiles due to differences in allergen exposure in Africa.[37] There was no evidence of anti-parasite IgE in this cohort of children. We previously showed that circulating anti-parasite IgE titres were strongly positively correlated with anti-mosquito saliva IgE, but became undetectable following malaria exposure, potentially being bound to effector cells.[31] Only mosquito saliva, a known major local

allergen, induced a specific IgE response at significantly higher titres in individuals with atopic dermatitis.

Although the immune effectors of clinical immunity are still poorly defined, there is strong evidence that acquired anti-parasite immunity is IgG-dependent[38] and cytophilic immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by opsonisation and/or by Antibody Dependent Cellular Immunity play an important role in premunition.[35] The higher parasite density during symptomatic episodes observed in the asthma group suggests impaired development of acquired immunity. Impaired acquisition of immunity to malaria in children with asthma or atopic dermatitis may stem from their imbalanced Th1/Th2 response. Indeed, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*. Dendritic cells that are oriented to a Th2 phenotype are more susceptible to orient the acquired immune response towards a Th2 profile.[39] Orientation of the immune response towards a Th2 profile by asthma or atopic dermatitis would result in a poor Th1 response (and hence development of protective IgG immunoglobulins), considered to be the dominant arm of the immune response enabling resistance to infectious disease in children.[40]

~~Such differences likely reflect the different IgE reactivity profiles due to differences in allergen exposure in Africa[25].~~

Many studies have revealed an important role of histamine, a key downstream effector molecule in allergic reaction, in the outcome of a malaria parasite infection.[22-24,~~11-13~~,41-44~~26 30~~] Moreover, reports indicate that components of the innate immune system, including eosinophils, basophils, and mast cells (MCs), could play important roles in the pathogenesis of malaria.[41~~26~~] Increased levels of histamine in plasma and tissue, derived from basophils and MCs, notably following stimulation by IgE through the high affinity receptor FcεR1, are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[23~~12~~,24~~13~~] Chlorpheniramine, a HR1agonist reversed resistance to chloroquine and amodiaquine both *in vivo* and *in vitro*.[42~~27~~] Moreover, astemizole, another HR1 agonist, was identified as an anti-malarial agent in a clinical drug library screen.[43~~28~~] Finally, *P. falciparum* produces translationally controlled tumor protein, which is a homolog of the mammalian histamine-releasing factor that causes histamine release from human basophils.[44~~29~~]

Further research

Our results provide the first birth cohort study addressing the link between malaria and allergic diseases. They contribute to a growing body of evidence that the pathologies are related. ISAAC has revealed a steady but significant increase in prevalence rates of asthma and allergic diseases in Africa. Whilst the majority of studies have focused on large cities, there is increasing urbanization throughout Africa, as well as improved access to primary health care in many areas. A key concern for ISAAC is the extent to which such societal evolution will result in an increase in allergic diseases. Increased urbanization in sub-Saharan Africa is changing the epidemiology of malaria and although resulting in a decrease in risk, will result in more severe clinical malaria in older individuals.[45,46] Moreover, a large consumption of anti-malarial drugs in the urban areas provides substantial drug pressure fostering, the selection of drug-resistant parasites. Despite the encouraging recent decrease in malaria incidence rates, even in rural areas, an additional significant concern is the extent to which such an increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines[47], administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials[48] and alleviate the non-infectious consequences of atopy. Clinical intervention studies should be envisaged.

What is already known on this topic

There are several reports of the beneficial effects of anti-histamines for malaria chemoprophylaxis[22-24,47] as well as our previous work[8,18] showing that chromosomal regions associated with malaria are also linked to allergy and atopy.[15-17] There are two epidemiological studies showing opposite effects of malaria on atopy.[20,36]

What this study adds

Using a longitudinal malaria study birth cohort, we identified an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred only after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

We are grateful to the villagers of Dielmo for their participation and sustained collaboration in this project as well as the field workers (Abdoulaye Badiane) and the data manager (Joseph Faye) for their contribution to the project and in generating and maintaining the malaria database. We are indebted to Michel Thiakane, a paediatric allergologist, head of the Paediatric Service, Principal hospital, Dakar, Senegal and Marie-Thérèse Guinnepain, dermatologist, Institut Pasteur clinic, Paris, France for aid in elaborating the ISAAC questionnaire and advising on clinical diagnosis of allergy in Senegal. We specially thank the children and parents or guardians who participated in the cross-sectional survey '*Pallergo*'. We thank the International Division of the Institut Pasteur that has given full support to this study.

STROBE: This article has been prepared according to the STROBE checklist guidelines.

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Contributors: LB, SM, and RP made substantial contributions to the concept and design of the study. MH, HB, BG, SB, FDS, and AT were involved in acquisition of the data. CL, AF, OMP, AS and RP contributed to the analysis and interpretation of the data. MH, CL, HB, BG, SB, FDS, AF, AT, LB, OMP, SM, AS and RP critically reviewed the report and approved its final version for submission. All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. MH and RP are guarantors.

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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Ethical approval: The allergy study was approved by the Senegalese National Ethics committee (2009/N°46). Renewed approval of the longitudinal malaria study was obtained from the same committee (2006/N°969).

Data sharing: The allergy database will be made available on-line. The longitudinal malaria data set will be made available following discussion with the coordinators of the three Institutes that govern the dataset through contact with the corresponding author.

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Table 1 Classification of Asthma, Allergic rhinoconjunctivitis, Atopic dermatitis and overall Atopic status according to ISAAC questionnaire in children aged 0-14 from a malaria birth cohort. N is total number of children examined and n-malaria represents those for whom malaria data were recorded. F is the number of females and M the number of males.

	N (F/M)	%	n-malaria (F/M)
Asthma symptoms			
None	153 (73/80)	87.43	125 (59/66)
Moderate	4 (1/3)	2.29	4 (1/3)
Severe	18 (6/12)	10.29	14 4/10)
Rhinoconjunctivitis symptoms			
None	146 (64/82)	83.43	120 (52/68)
Moderate	11 (8/3)	6.29	9(6/3)
Severe	18 (6/12)	10.29	14 (6/8)
Atopic dermatitis symptoms			
None	159 (75/84)	90.86	128 (60/68)
Moderate	11 (1/10)	6.29	11 (1/10)
Severe	5 (4/1)	2.86	4 (3/1)
Atopic tendency			
Unlikely	119 (56/63)	68.00	97 (46/51)
Probable	16 (8/8)	9.14	14 (6/8)
Highly probable	40 (16/24)	22.86	32 (12/20)

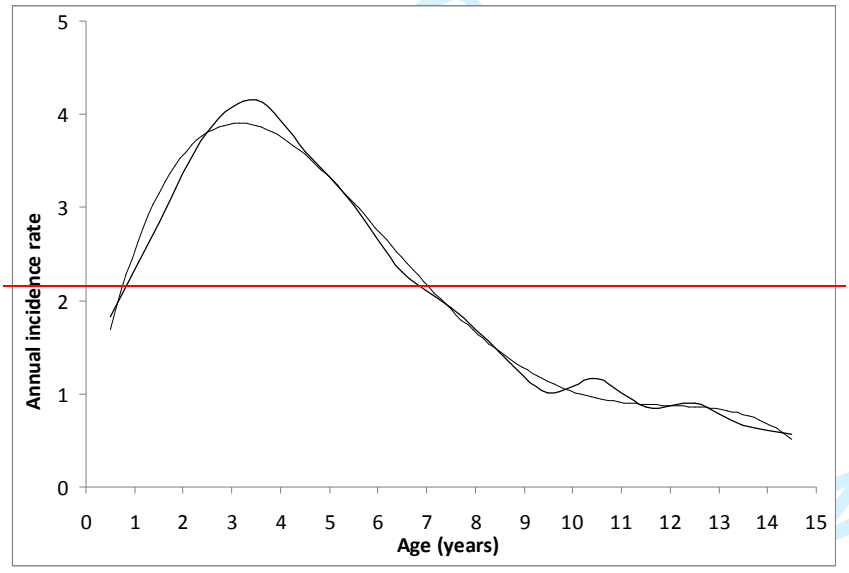
Table 2 Impact of allergy status on risk of *P. falciparum* clinical episodes. Shown are the *P* values and adjusted Odds Ratios with 95% confidence intervals calculated from the mixed model analyses. Values for the covariables Age (≥3.-5 years of age compared to <3.-5 years of age), Trimestrial incidence of *P. falciparum* clinical episodes and HbAS (beta-globin sickle cell trait; AS compared to AA) are those from the Asthma model analysis. For clarity significant co-variables are shown in bold.

	Age groups <3.-5 years>	ORa	95% Confidence Intervals		*P* value
			Lower	Upper	
Atopy	**Both**	**1.65**	**1.20**	**2.26**	**2.0×10^{-3}**
	<3.5	1.38	0.92	2.08	0.124
	≥3.5	**2.02**	**1.39**	**2.93**	**2.1×10^{-4}**
Asthma	**Both**	**2.12**	**1.46**	**3.08**	**8.0×10^{-5}**
	<3.5	1.50	0.90	2.50	0.122
	≥3.5	**2.33**	**1.50**	**3.61**	**1.5×10^{-4}**
Atopic dermatitis	Both	1.05	0.65	1.70	0.842
	<3.5	0.84	0.49	1.46	0.539
	≥3.5	**3.15**	**1.56**	**6.33**	**1.3×10^{-3}**
Rhinoconjunctivitis	Both	0.96	0.65	1.41	0.818
	<3.5	1.05	0.64	1.72	0.853
	≥3.5	0.95	0.60	1.52	0.834
Age ≥3.5		**0.48**	**0.40**	**0.57**	**2.7×10^{-15}**
Trimestrial incidence		**1.01**	**1.00**	**1.01**	**1.8×10^{-6}**
HbAS		**0.24**	**0.12**	**0.47**	**3.7×10^{-5}**

Table 3 Impact of allergy status on the maximum *P. falciparum* parasite density during a clinical malaria episode. Shown are the back-transformed mean parasite densities per microlitre and standard errors (SEM) estimated from the GLMM analyses after taking into account the other co-variables. Significantly different effects are shown in bold for clarity.

Allergic condition	Age groups	Allergic status (No/Yes)	Mean parasite density	SEM	*P* value
Atopy	**Both**	**N**	**76.3**	13.8	
		Y	**131.0**	36.4	**0.0158**
	<3.5	N	114.3	23.7	
		Y	171.1	56.0	0.148
	≥3.5	**N**	**48.4**	9.8	
		Y	**114.8**	37.1	**$9.5x10^{-4}$**
Asthma	**Both**	**N**	**78.1**	14.4	
		Y	**148.5**	44.3	**$3.8\ x10^{-3}$**
	<3.5	N	114.8	24.3	
		Y	171.9	74.5	0.167
	≥3.5	**N**	**51.3**	9.7	
		Y	**105.3**	41.0	**$6.2\ x10^{-3}$**
Atopic dermatitis	Both	N	82.6	15.0	
		Y	93.9	38.9	0.605
	<3.5	N	122.6	25.5	
		Y	133.9	63.5	0.425
	≥3.5	**N**	**52.3**	11.0	
		Y	**135.4**	70.7	**0.014**
Rhinoconjunctivitis	Both	N	81.5	14.8	
		Y	111.4	39.0	0.570
	<3.5	N	118.8	25.1	
		Y	166.3	69.9	0.537
	≥3.5	N	54.6	11.3	
		Y	80.9	33.7	0.327

Figure 1 Annual incidence rate of clinical *P. falciparum* episodes per 100 children (~~solid~~ bold line). In order to overcome the fluctuations of the annual incidence rate, we fit second (dotted line), third (dashed line) and fourth (solid line) degree polynomial trend lines to the data (bold line). The corresponding R-squared values are 0.70, 0.91 and 0·99 respectively indicating an accurate fit for third and fourth order polynomials. The inflexion on these two trend lines indicates the onset of acquisition of clinical immunity at approximately 3 to 4 years of age.~~a fourth degree polynomial trendline to the data (dashed line). The corresponding R-squared value is 0·9855 indicating an accurate fit. The inflexion on the trendline indicates the onset of acquisition of clinical immunity at approximately 3·5 years of age.~~



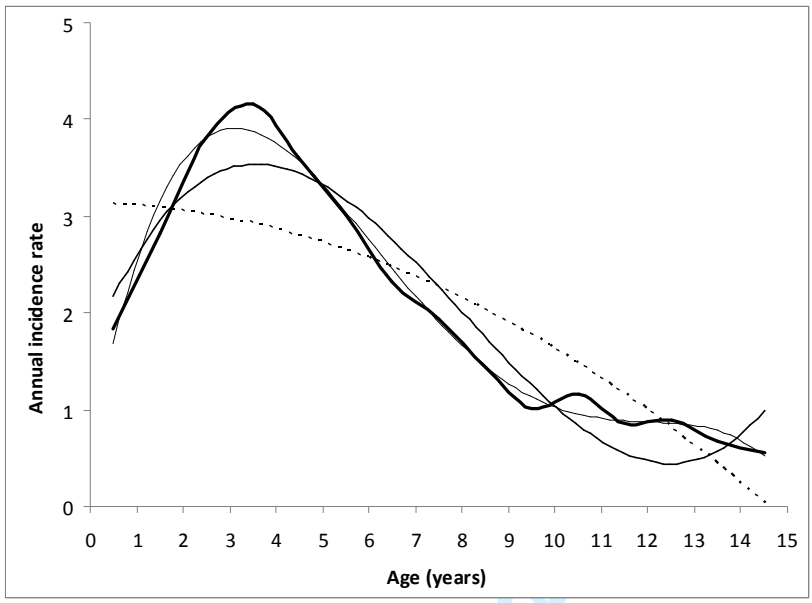
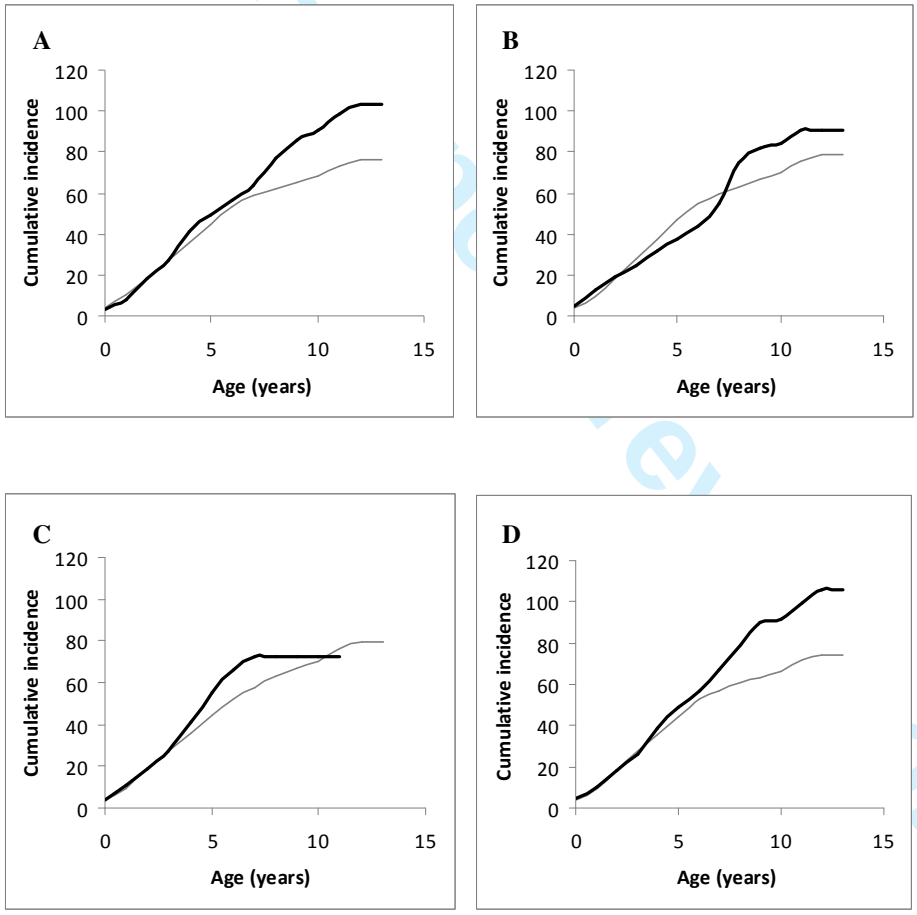


Figure 2 Mean cumulative number of *P. falciparum* clinical episodes with age for the (A) Asthma, (B) Rhinoconjunctivitis and (C) Atopic dermatitis classes and overall Atopy class (D) (bold lines) compared to individuals without symptoms of each respective allergy type (~~fine~~ thin lines). In all cases moderate and severe classes are combined and compared to indivi~~i~~duals without allergy symptoms. Note there are no children older than 11 years of age with Atopic dermatitis.



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Place of study	***Research Institute responsible***
Technician X	*Name of Institute*
Technician X	*Principle investigator*
Technician X	*Project manager*
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IDENTIFICATION

Validation zone

Date of questionnaire : |__|__| |__|__| |__|__|__|__| dd/*mm/yyyy* — |__|__| |__|__| |__|__|__|__| DTEQUE

Name of investigator :……

Name of study supervisor :……

Child :

First and last name of child :

Identification code of child : — |__|__| |__|__| IDENF

Date of birth : — |__|__| |__|__| |__|__|__|__| DOB

Sex : — |__| SEXE

Village/town : — |__| VILLAGE

Identification code of Questionnaire : — |__| |__|__| |__| |__|__||__|__| IDQUES

Identification code of father : — |__|__| |__|__| IDFA

Identification code of mother : — |__|__| |__|__| IDMO

Weight : |__|__|,|__| **(kg)** — |__|__|,|__| (Kg) WEIGHT

Height : |__|__|__|,|__| **(cm)** — |__|__|__|,|__| (cm) HEIGHT

Mid Upper Arm Circumference: |__|__| **(cm)** — |__|__| (cm) MUAC

Person questioned :

Last name of person questioned :…… — …… NAMEPQ

First name of person questioned :…… — …… LASTNAMEPQ

Relationship to child : Mother ☐1 Father ☐2 Brother/Sister ☐3 Grand-parents ☐4 Other ☐5 — |__| RELCHILD

If other, define : …… — …… OTHERREL

FACTORS PREDISPOSING ATOPY

First days of life : Consultation of health records of child and maternity records of mother

1. How much did your child weigh at birth ?

<1500 g ☐1 — [2500-3500[g ☐4

[1500-2000[g ☐2 — ≥ 3500 g ☐5

[2000-2500[g ☐3 — Record not found/NSP ☐9

|__| BIRTHWEIGH

2. Until what age did your child breastfeed (**exclusively or mixed**) ?
Corresponds to the age of weaning of child

< 6 months ☐1 6 – 12 mths ☐2 12 – 24 mths ☐3 >24 mths ☐4 NSP ☐9

|__| AGEWEAN

3. Until what age did your child breastfeed **exclusively** without ever taking other aliments (fruits, vegetables, rice, meat, fish, etc.) or liquids (powdered milk, cow or goats milk, fruit juice, water, etc.) ? < 6 months ☐$_1$ 6 – 12 mths ☐$_2$ 12 – 24 mths ☐$_3$ NSP ☐$_9$	|__|	AGEBREAST
Illness and vaccination : Consultation of health records of child		
1. Has your child enfant had the following illnesses?		
Malaria : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	MALAR
Tuberculosis treated : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	TUBTRT
Helminths (oxyures, ascaris, taenia, etc.) : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	HEMINTH
Amoeba : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	AMOEBA
Measles : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	MEASLES
2. Against what illnesses is you child vaccinated?		
Yellow fever : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACFJ
Hepatitis B : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACHEPB
Measles : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACMEASLE
Mumps : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACMUMPS
Rubella : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACRUBEL
Tuberculosis/BCG : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACTUB
Diphtheria/Tetanus/Pertussis/Poliomyelitis : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACDTCP
Typhoid : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACTY
Meningitis : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACMENIN
Haemophilus influenzae type B (HiB) : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACHIB
Habitation :		
1. Which of these animals / insects can be found in the **rooms** where your child lives (today and/or during his first year of life) ?		
Dogs in rooms today : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	DOGTODAY
Dogs in rooms 0-1yr : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	DOG01YR
Cats in rooms today : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	CATTODAY
Cats in rooms 0-1yr : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	CAT01YR
Sheep in rooms today : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	SHEEPTODAY
Sheep in rooms 0-1yr : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	SHEEP01YR
Goats in rooms today : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	GOATODAY
Goats in rooms 0-1yr : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	GOA01YR
Chicken, ducks in rooms today : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	CHICTODAY
Chicken, ducks in rooms 0-1yr : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	CHIC01YR
Rodents (rats, mice, etc.) in rooms today : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	RODTODAY
Rodents (rats, mice, etc.) in rooms 0-1yr : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	ROD01YR
Cockroaches in rooms today : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	COCTODAY
Cockroaches in rooms 0-1yr : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	COC01YR
Other in rooms today : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	OTHTODAY
Other in rooms 0-1yr : ☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	OTH01YR
If Others, define :……	……	NAMEOTH
2. Which of these animals could be in **contact** with your child **at least once per week**		

(today and/or during his first year of life) ?

Contact with Dogs today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CDOGTODAY
Contact with Dogs 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CDOG01YR
Contact with Cats today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCATODAY
Contact with Cats 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCAT01YR
Contact with Sheep today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CSHEEPTODAY
Contact with Sheep 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CSHEEP01YR
Contact with Goats today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CGOATODAY
Contact with Goats 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CGOA01YR
Contact with Chicken, Ducks today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCHICTODAY
Contact with Chicken, Ducks 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCHIC01YR
Contact with donkeys, horses today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CHORSTODAY
Contact with donkeys, horses 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CHORS01YR
Contact with Cows, zébus today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCOWTODAY
Contact with Cows, zébus 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCOW01YR
Contact with Rodents (rats, mice, etc.) today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CRODTODAY
Contact with Rodents (rats, mice, etc.) 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CROD01YR
Contact with Other today :	☐0 No ☐1 Yes ☐9 NSP	|__|	COTHTODAY
Contact with Other 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	COTH01YR
If Others, define :……		……	NAMEOTHC

3. Which of these aliments are usually stocked in the rooms where your child lives ?

Millet kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	MIL
Sorghum kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	SORG
Maize kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	MAIZ
Rice kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	RICE
Wheat kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	WHEA
Biscuits, pasta kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	BISCUI
Manioc (root, flour) kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	MANIOC
Cashew nut, ground nut kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	NUTP
Curdled milk kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	MILKCURD
Dried leaves (mint, quinquiliba, baobab, etc.) :	☐0 No ☐1 Yes ☐9 NSP	|__|	LEAF
Other aliments kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	OTHALIM
If Others, define :……		……	NAMEOTHAL

What is the type of roofing of the rooms where your child lives (today and during the first year of life) ?

Corrugated metal roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	RMETTODAY
Corrugated metal roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RMET01YR
Thatched roof today:	☐0 No ☐1 Yes ☐9 NSP	|__|	RTHATDAY
Thatched roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RTHAT01YR
Wooden roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	RWOOTODAY
Wooden roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RWOO01YR
Cement roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	RCEMTODAY
Cement roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RCEM01YR
Plaster roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	RPLATODAY
Plaster roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RPLA01YR
Other type of roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	ROTHTODAY

Other type of roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| ROTH01YR
If other, define :.....		..... NAMEOTHR

4. Which of these objects are in the room where your child sleeps (today and during the first year of life) ?

Mattress in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| MATRTODAY
Mattress in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| MATR01YR
Bednet in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| BEDNTODAY
Bednet in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| BEDN01YR
Wardrobe in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| WARDTODAY
Wardrobe in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| WARD01YR
Chest, trunk in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| CHESTODAY
Chest, trunk in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| CHES01YR
Table in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| TABPTODAY
Table in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| TABP01YR
Chair in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| CHPTODAY
Chair in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| CHA01YR
Carpet, rug in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| CARPTODAY
Carpet, rug in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| CARP01YR
Matting in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| MATPTODAY
Matting in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| MATP01YR
Curtains in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| CURTTODAY
Curtains in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| CURT01YR
Malagasy fire in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| FIRTODAY
Malagasy fire in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| FIR01YR
Other objects in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| OTHOBTODAY
Other objects in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| OTHOB01YR
If other, define :.....		..... NAMEOTHOB

5. On what type of bedding does your child sleep (today and during the first year of life) ?

Foam mattress today :	☐0 No ☐1 Yes ☐9 NSP	|__| FMATRTODAY
Foam mattress 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| FMATR01YR
Plant fibre mattress (straw, etc.) today :	☐0 No ☐1 Yes ☐9 NSP	|__| PLFMATRTODAY
Plant fibre mattress (straw, etc.) 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| PLFMATR01YR
Wool mattress today :	☐0 No ☐1 Yes ☐9 NSP	|__| WOMATRTODAY
Wool mattress 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| WOMATR01YR
Feather mattress today :	☐0 No ☐1 Yes ☐9 NSP	|__| FEATHMTODAY
Feather mattress 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| FEATHM01YR
Plastic matting today :	☐0 No ☐1 Yes ☐9 NSP	|__| PLMATTODAY
Plastic matting 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| PLMAT01YR
Plant fibre matting (straw, etc.) today :	☐0 No ☐1 Yes ☐9 NSP	|__| PLFMATTODAY
Plant fibre matting (straw, etc.) 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| PLFMAT01YR
Other type of bedding today :	☐0 No ☐1 Yes ☐9 NSP	|__| OTHBEDTODAY
Other type of bedding 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| OTHBED01YR
If other, define :.....		..... NOMAUTLI

6. Does your child sleep on a pillow ? ☐0 No ☐1 Yes ☐9 NSP \|__\| PILLOW

If **No**, go to question **8**

If **Yes**, what type of pillow is it ?

Foam :	☐0 No ☐1 Yes ☐9 NSP	|__|	PILLF
Synthetic fibres:	☐0 No ☐1 Yes ☐9 NSP	|__|	PILLSYN
Plant fibres (straw, etc.) :	☐0 No ☐1 Yes ☐9 NSP	|__|	PILLPLF
Feather :	☐0 No ☐1 Yes ☐9 NSP	|__|	PILLFEATH
Other type of pillow :	☐0 No ☐1 Yes ☐9 NSP	|__|	OTHPILL
If other, define :.....		.....	NAMEOTHPILL

7. Do people smoke in the room where your child lives ?

Today :	☐0 No ☐1 Yes ☐9 NSP	|__|	SMOKTODAY
From 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	SMOK01YR
During the pregnancy of the mother :	☐0 No ☐1 Yes ☐9 NSP	|__|	SMOKPREG

8. What type of heating and lighting are used in the rooms where your child lives ?

Heating and lighting by charcoal :	☐0 No ☐1 Yes ☐9 NSP	|__|	CHELCHAR
Heating and lighting by wood :	☐0 No ☐1 Yes ☐9 NSP	|__|	CHELWOO
Lighting by candle :	☐0 No ☐1 Yes ☐9 NSP	|__|	LCAND
Lighting by petrol lamp :	☐0 No ☐1 Yes ☐9 NSP	|__|	LLAMP
Lighting by flash light :	☐0 No ☐1 Yes ☐9 NSP	|__|	LTORCH
Lighting by solar :	☐0 No ☐1 Yes ☐9 NSP	|__|	LSOLAR
Other types of heating and lighting:	☐0 No ☐1 Yes ☐9 NSP	|__|	OTHHEL
If other, define :.....		.....	NAMEOTHHEL

9. Which of the following products are used or stocked in the rooms where you child lives ?

Insecticide (type Yotox, spirales, etc.) :	☐0 No ☐1 Yes ☐9 NSP	|__|	INSECTIC
Deodorants (aerosols) :	☐0 No ☐1 Yes ☐9 NSP	|__|	DEODORA
Incense :	☐0 No ☐1 Yes ☐9 NSP	|__|	INCENSE
Detergents (type Cotol, etc.) :	☐0 No ☐1 Yes ☐9 NSP	|__|	DETERGEN
Petrol, diesel :	☐0 No ☐1 Yes ☐9 NSP	|__|	PETROL
Other types of products :	☐0 No ☐1 Yes ☐9 NSP	|__|	OTHPROD
If other, define :.....		.....	NAMEOTHPR

Diet :

1. Has your child had **diarrhoea without fever** or abdominal pains (colic)

following introduction of non-maternal milk in his diet (cow or goat's milk, milk powder) :	☐0 No ☐1 Yes ☐9 NSP	|__|	DIARINT
after a few months of consuming **non-maternal** (cow or goat's milk, milk powder) :	☐0 No ☐1 Yes ☐9 NSP	|__|	DIARMONTH

2. Currently, how many times, on average, does your child eat the following aliments ?

The consumption of certain aliments is seasonal.

Meat :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMEAT
Fish :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSFISH
Egg :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSEGG
Milk (liquid, powder, curdled) :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMILK
Banana :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSBANA
Mango :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMANG
Melon :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMELON

Orange, lime :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSORAN
Potatoes, sweet potatoes :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSPOT
Vegetables :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSVEG
Millet :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMIL
Sorghum :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSSORG
Maize :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMAIS
Rice :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSRICE
Wheat (bread, pasta) :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSWHEA
Nuts (Cashew, ground nut) :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSNUT
Prawns, dried oysters :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSPRAWN
Flavouring cubes Maggi :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSCUBE
Other :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	OTHALCON
	If other, define :.....	.....	NAMEOTHAL

HISTORICAL SYMPTOMATOLOGY OF ALLERGIC REACTIONS

Asthma :

1. Has a doctor or nurse **already** said that you child has asthma ?

☐0 No ☐1 Yes ☐9 NSP — \|__\| ASTHMA

2. Has your child already breathed noisily or had whistling in his chest whilst breathing

☐0 No ☐1 Yes ☐9 NSP

If **No**, go directly to question **6**

\|__\| WHISTLING

3. During his first two years of life, has your child already breathed noisily or had whistling in his chest whilst breathing ?

☐0 No ☐1 Yes ☐9 NSP — \|__\| WHISTL2YR

If **No**, go directly to question **6**

If **Yes**, how many times (before 2 years of age) ?

☐1 1time ☐2 2times ☐3 ≥3times ☐9 NSP — \|__\| NBWHIS2YR

Between the last two **ramadans**, has your child already breathed noisily or had whistling in his chest whilst breathing ?

☐0 No ☐1 Yes ☐9 NSP — \|__\| WHISTL2RA

If **No**, go directly to question **5**

If **Yes**, at which moment of the year ?

Rainy season :	☐0 No ☐1 Yes ☐9 NSP	|__| WHISTLRS
Dry season :	☐0 No ☐1 Yes ☐9 NSP	|__| WHISTLDS
Harvest time :	☐0 No ☐1 Yes ☐9 NSP	|__| WHISTLHT

Has the noisy breathing of your child been such that it has prevented him from talking normally?

☐0 No ☐1 Yes ☐9 NSP — \|__\| PREVTALK

Has your child already had a rasping cough at night that prevents him from sleeping normally ?

☐0 No ☐1 Yes ☐9 NSP — \|__\| TOUSECHE

Rhinitis and allergic conjunctivitis;

1. Has your child **already had** problems of a runny nose, **or** a sensation of a blocked nose, **or** an itchy nose, **or** sneezing, or loss of the sense of smell **for more than a week,**

irrespective of the frequency of these episodes? ☐0 No ☐1 Yes ☐9 NSP	|__|	RHIN1WEEK
2. Has your child **already had** problems of a runny nose, **or** a sensation of a blocked nose, **or** an itchy nose, **or** sneezing, or loss of the sense of smell **more than 5 times in one year,** irrespective of the frequency of these episodes? ☐0 No ☐1 Yes ☐9 NSP	|__|	RHIN5FAN
Between the **last two ramadans**, has your child **already had** problems of a runny nose, **or** a sensation of a blocked nose, **or** an itchy nose, **or** sneezing, or loss of the sense of smell ? ☐0 No ☐1 Yes ☐9 NSP	|__|	RHIN2RAM
If **No**, go to question **4**		
If **Yes**, at what moment of the year ?		
Rainy season : ☐0 No ☐1 Yes ☐9 NSP	|__|	RHINRS
Dry season : ☐0 No ☐1 Yes ☐9 NSP	|__|	RHINDS
Harvest time : ☐0 No ☐1 Yes ☐9 NSP	|__|	RHINHT
3. Has your child **already had** watery eyes, **or** itchy eyes, **or** an allergic limbo-conjonctivitis? ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJALER
If **No**, go directly to question **1** in the section **Eczema**		
Has your child had, between the **last two ramadans**, watery eyes, **or** itchy eyes, **or** an allergic limbo-conjonctivitis? ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJ2RAM
If **No**, go directly to question **5**		
If **Yes**, at what moment of the year ?		
Rainy season : ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJRS
Dry season : ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJDS
Harvest time : ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJHT
Eczéma :		
Has your child **already had** skin problems with dry patches or seeping cracked patches and itching ? ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEMA
If **No**, **the questionnaire has finished.**		
Between the **last two ramadans**, has your child had skin problems with dry patches or seeping cracked patches and itching ?? ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZE2RAM
If **No**, go directly to question **3**		
If **Yes**, at what moment of the year ?		
Rainy season : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEMARS
Dry season : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEMADS
Harvest time : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEMAHT
1. Have these skin problems affected different parts of the body of your child ?		
Scalp : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZESCALP
Face : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEFAC
Around the eyes and ears : ☐0 No ☐1 Yes ☐9 NSP		
Armpits : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEEYEEAR

Elbow :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEARMPIT
Hands :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEELBOW
Under the buttocks:	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEHAND
Groin :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEBUTT
Behind the knee :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEGROIN
Feet :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEKNEE
Other part of body :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEFEET
		|__| ECZEOTH

What age did your child have when these skin problems of dry patches, weeping cracked patches or itching appear for the **first time**?

☐1 < 2yr ☐2 2 - 4 yr ☐3 ≥ 5yr

\|__\| AGECZEMA

Have your child's skin problems ever been sufficiently important to prevent him from sleeping correctly or waking him up during the night ?

☐0 No ☐1 Yes ☐9 NSP

\|__\| IRRECZEM

Comments : Note with reference to which questions these comments apply

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STROBE Statement—checklist of items included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Asthma and atopic dermatitis are associated with increased risk of clinical Plasmodium falciparum malaria

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Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium falciparum* malaria

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Article summary

Article focus

- Genetic studies suggest a link between susceptibility to allergy and malaria in Africa
- We hypothesize that atopy increases susceptibility to malaria

Key messages

- Results demonstrate an association between asthma, atopic dermatitis and susceptibility to clinical *P. falciparum* episodes.
- Genetic pre-disposition to asthma or atopic dermatitis impairs the acquisition of clinical immunity to malaria.
- Administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Strengths and limitations

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association.

Abstract

Objectives: To assess the impact of atopy and allergy on the risk of clinical malaria.

Design: A clinical and immunological allergy cross-sectional survey in a birth cohort of 175 children from 1 month to 14 years of age followed for up to 15 years in a longitudinal open cohort study of malaria in Senegal. Malaria incidence data were available for 143 of these children (aged 4 months to 14 years of age) for up to 15 years. Mixed model regression analysis was used to determine the impact of allergy status on malaria incidence, adjusting for age, gender, sickle cell trait and force of infection.

Main outcome measures: Asthma, allergic rhinoconjunctivitis and atopic dermatitis status, the number of clinical *Plasmodium falciparum* malaria episodes since birth and associated parasite density.

Results: Twelve percent of the children were classified as asthmatic and ten percent as having atopic dermatitis. These groups had respectively a two-fold (OR 2.12 95% confidence intervals 1.46 to 3.08; P= 8 $x10^{-5}$) and three-fold (OR 3.15, 1.56 to 6.33; P= 1.3 $x10^{-3}$) increase in the risk of clinical *P. falciparum* malaria once older than the age of peak incidence of clinical malaria (3 to 4 years of age). They also presented with higher *P. falciparum* parasite densities (Asthma: mean 105.3 parasites/μL ±SE 41.0 *vs*. 51.3 ±9.7; P= 6.2 $x10^{-3}$; Atopic dermatitis: 135.4 ±70.7 *vs*. 52.3 ±11.0; P=0.014). There was no effect of allergy on the number of non-malaria clinical presentations. Individuals with allergic rhinoconjunctivitis did not have an increased risk of clinical malaria nor any difference in parasite densities.

Conclusion: These results demonstrate that asthma and atopic dermatitis delay the development of clinical immunity to *P. falciparum*. Despite the encouraging decrease in malaria incidence rates in Africa, a significant concern is the extent to which the increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines, administration to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Introduction

The World Allergy Organization estimates that 40% of the world's population is concerned by allergic diseases.[1] In developing countries where *Plasmodium falciparum* malaria is endemic, prevalence of allergy is significantly lower, but is on the increase.[2] T helper type 2 (Th2) cells, their related cytokines, IgE, eosinophils and mast cells play a major role in allergic inflammation. Orientation of the immune response towards a Th1 profile is crucial for immunity to intracellular pathogens,[3] whereas orientation towards a Th2 profile drives immunity to extracellular pathogens and antigens resulting in class switching giving rise to IgE-producing B cells.[4] A role of the Th1/Th2 balance in the development of clinical malaria following infection by *P. falciparum* has been suggested by numerous studies.[5-7] Whilst it is recognised that acquired anti-parasite immunity is IgG dependent,[8] parasite-specific IgE also impact upon the clinical outcome of infection. For example, higher IgE but not IgG levels have been observed in patients with cerebral malaria than those with uncomplicated P. falciparum infection.[9] The role of IgE, however, remains unclear. [10]

The interplay between infectious agents and allergy is ambiguous. On the one hand, for example, severe respiratory syncytial virus infection in infants increased the risk of allergic rhinoconjunctivitis and allergic asthma.[11,12] On the other hand, measles,[13] hepatitis A[14] and tuberculosis[15] seemingly reduce atopy. Although, an atopic condition can increase incidence of disease, such as the case for the skin commensal *Staphylococcus aureus* in patients with atopic dermatitis,[16] an atopic tendency *per se* does not generally lead to increased illness from infectious agents.

Genome wide studies have identified chromosomal regions linked to clinical malaria, all of which overlap with those previously identified to be involved in atopic dermatitis, asthma, atopy and IgE levels,[17-19] suggesting that common mechanisms may be involved in both pathologies.[20] Chromosomal region 5q31 that has been repeatedly shown to be associated with control of parasite density and contains a cluster of cytokines, among which IL12B has been previously associated with psoriasis.[21] The other regions, 13q13-q22, 5p15-p13 and 12q21-q23, contain genes involved in innate immunity, notably the interleukin 7 receptor, and several involved in tumour necrosis factor synthesis [C1q and tumour necrosis factor related protein 3 (C1QTNF3)] and a gene involved in the complement system (C9). [20]

Several additional lines of evidence support the concept that susceptibility to malaria and atopy may be related to similar immunological defects. In Ethiopia, a history of malaria was associated with atopy.[22] A mouse model for human atopic disease was found to be very susceptible to murine malaria and a major locus for atopic disease mapped close to the region controlling parasite density.[23] This region contains several candidate genes that have effects on T-cell function.[23]

Moreover, a direct effect of histamine in the malaria pathogenesis has been found using genetic and pharmacological approaches[24] and increased levels of histamine are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[25,26]

To test the hypothesis that allergy impacts upon clinical *P. falciparum* malaria, we performed a clinical allergy cross-sectional study in the family-based longitudinal cohort from Senegal previously used for the genome linkage study[20] and analysed the impact of asthma, atopic dermatitis, allergic rhinoconjunctivitis on the incidence of clinical *P. falciparum* episodes and the maximum parasite density during each episode.

Methods

Population and outcome data

The malaria research program conducted in Dielmo village in Senegal has been ongoing since 1990 as described elsewhere.[27] In brief, between 1990 and 2008, a longitudinal study involving the inhabitants of the village of Dielmo, Senegal, was carried out to identify all episodes of fever. The study design included daily medical surveillance with systematic blood testing of individuals with fever and examination of 200 oil-immersion fields on a thick blood film for malaria parasites (about 0.5 µL of blood). Each individual was given a unique identification code and details of family ties, occupation, and precise place of residence were recorded on detailed maps of each household with the location of each bedroom. All households were visited daily, absenteeism recorded, and the presence of fever or other symptoms assessed. We systematically recorded body temperature at home three times a week (every second day) in children younger than 5 years, and in older children and adults in cases of suspected fever or fever-related symptoms. In cases of fever or other symptoms,

blood testing was done at the dispensary by finger prick, and we provided detailed medical examination and specific treatment. Parasitologically confirmed clinical malaria episodes were treated according to national guidelines. From 1990 to 2008, four different drug regimens were implemented: Quinine from 1990 to 1994, Chloroquine from 1995 to 2003, Fansidar (sulfadoxine-pyrimethamine) from 2004 to mid-2006 and Artemisinin-based combination therapy (ACT; Amodiaquine- sulfadoxine-pyrimethamine) from mid-2006 to 2008.

Parasite positivity was established as follows. Thick blood films were prepared and stained by 3% Giemsa stain. Blood films were examined under an oil immersion objective at x1000 magnification by the trained laboratory technicians and 200 thick film fields were examined to count the number of asexual and gametocyte parasite stages. Asexual parasite densities (per µL) were calculated by establishing the ratio of parasites to white blood cells and then multiplying the parasite count by 8,000, the average white blood cell count per µL of blood.

Malaria transmission in Dielmo is intense and perennial. We conducted a cross-sectional survey to estimate the prevalence of symptoms related to allergic diseases among 175 children aged from 1 month to 14 years old who were born during the malaria research program.

Both the longitudinal and cross-sectional surveys were approved by the Ministry of Health of Senegal. Informed consent of the volunteers is renewed every year. More specifically for the cross-sectional survey, after informing about the procedures and the purpose of the study, written informed consent was obtained from parents or guardians of children either by signature or by thumbprint on a voluntary consent form written in both French and Wolof, the main local language. Consent was obtained in the presence of the school director, an independent witness.

The family structure (pedigree) was available after a demographic census performed for every volunteer at his adhesion in the project. A verbal interview of mothers or key representatives of the household was used to obtain information on genetic relationships between studied individuals, their children, their parents, and to identify genetic links among the population. The total pedigree comprised 828 individuals, including absent or dead relatives, composed of ten independent families that can be sub-divided into 206 nuclear families (father – mother couples with at least one child) with an average of 3.6

children each. Genetically related nuclear families occur because of multiple marriages and marriages among related individuals. Previous typing with microsatellites has enabled the construction of a pedigree based on Identity-by-Descent using MERLIN.[20,28] The mean coefficient of inbreeding is 0.0008. Newborns since this original genetic analysis were added to the family of the parents in question. The 143 children, with both allergy and malaria data, belonged to 61 nuclear families and comprised 30 singletons, 102 siblings and 11 half-sibs (yielding 55 half-sib pairs). The mean genetic relatedness (by pedigree) of the 143 children is 0.0114 (range: 0.0013 to 0.022).

P. falciparum *clinical episodes*

P. falciparum malaria clinical episode phenotypes analysed were: (i) clinical *P. falciparum* infections treated with anti-malarial therapy and (ii) the highest parasite density during the *P. falciparum* clinical episode. A clinical *P. falciparum* episode was defined as a clinical presentation with fever (axillary temperature ≥ 37.5°C) and/or other clinical signs suggestive of malaria associated with a thick blood smear positive for *P. falciparum* and that was treated with anti-malarial therapy. Repeated clinical malaria presentations within 15 consecutive days were not considered to be independent and were excluded from the analyses, unless there was a negative thick blood smear between two clinical presentations. We also excluded observations in any trimester for which the individual was not present for at least one third of the time.

We calculated the quarterly incidence rate of clinical *P. falciparum* episodes in children below the age of 15 years as the ratio of the total number of clinical *P. falciparum* episodes during the trimester divided by the total number of person-trimesters surveyed. Incidence rate is expressed as cases per 100 person-trimesters (see Supplementary Figure S1). This rate was used in the analysis to approximate the force of infection (exposure level) within the targeted population at the time of a given clinical *P. falciparum* episode.

The total number of clinical presentations per trimester that were not attributable to *P. falciparum* was tabulated. Repeated non-malaria presentations within seven consecutive days were not considered to be independent and were excluded.

Allergic diseases and atopic status

The International Study of Asthma and Allergies in Childhood (ISAAC) diagnostic criteria have been shown to be reproducible, adequate and able to discriminate children with allergic diseases in different areas of the world.[2] The standardized ISAAC questionnaire originally written in English was translated into French in compliance with ISAAC guidelines[29], adapting it to the usual local customs following advice from local clinicians and paediatric allergologists (Acknowledgements and Technical Appendix). The adequacy and reliability of the translated questionnaire had been previously confirmed by a pilot study on 30 randomly selected children in the same community. The questionnaire was completed by specially trained health workers during an oral interview conducted in Wolof with children and their mothers or guardians.

To assess the prevalence of allergic diseases in children, we used the positive and negative predictive values of the ISAAC questionnaire diagnosis criteria developed for subtropical countries.[30] Each question was scored according to the medical diagnosis of paediatricians and paediatric allergologists. Positive or negative answers were thus graded on the basis of symptom sensitivity, specificity, frequency, location or early onset. For each allergic disease, three categories of symptom severity, *severe*, *moderate*, and *none*, were defined as follows:

Asthma – severe symptoms if the child had "wheezing or whistling in the chest before the age of two years" and "more than three times" *or* severe enough to "limit his/her speech"; *moderate* symptoms if the child had "wheezing or whistling in the chest before the age of two years" and "in the past 12 months"; and *none* otherwise.

Allergic rhinoconjunctivitis – severe symptoms if the child had "sneezing, runny or stuffy nose in the past 12 months" and "more than five times a year", and "itchy, watery eyes or tropical endemic limboconjunctivitis (TELC) in the past 12 months"; *moderate* symptoms if the child had "sneezing, runny or stuffy nose in the past 12 months", and "itchy, watery eyes or TELC in the past 12 months"; and *none* otherwise.

Atopic dermatitis – severe symptoms if the child had "scaly or exudating, crusted and pruritic patches in the past 12 months" and "affecting any of the following characteristic areas: face, around the ears or eyes, folds of armpits or elbows or groin, behind the knees, under the buttocks", and "onset of symptoms before the age of two years"; *moderate* symptoms if the child had "scaly or exudating, crusted and pruritic patches in the past 12 months" and

"affecting any of characteristic areas (see above)", and "onset of symptoms before the age of four years"; and *none* otherwise.

The inter-relationships between variables reflecting the severity of symptoms of the three allergic diseases were used to identify children at high risk of atopy. The *high probability* group was defined by the prevalence of at least one of any *severe* symptoms or two of any *moderate* symptoms. The *probable* group was defined as those with *moderate* symptoms from one of the three allergic diseases and remaining children were classified in the *unlikely* group.

Helminths

Helminthic infections are common in this region and are known to modify the clinical course and outcome of both allergic diseases and malaria.[31,32] We therefore carried out a helminth survey for 91 individuals present during the cross-sectional survey. Diagnosis was performed by stool examination by microscope and by the Kato technique to search for the presence of *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*), whipworm (*Trichuris trichiuria*), *Schistosoma mansoni*, and *Strongyloides stercoralis*. Examination for pinworms (*Enterobius vermicularis*) was performed by the anal scotch-test. An anti-helminthic treatment was proposed for all infested individuals.

Immunoglobulin E titres

Specific IgE titres were measured by ELISA as previously described.[33] A panel of allergens of potential pertinence to the three classes of allergy was used: (i) Salivary gland extracts (SGE) of two mosquito species present in the study cohorts, *Aedes aegypti* and *Anopheles gambiae sensu stricto*, and (ii) *P. falciparum* parasite extract were prepared as previously described[31]; (iii) House dust mite spp. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*; (iv) a mix of pollen allergens from five ubiquitous graminae spp. [Cock's-foot (*Dactylis glomerata*), Timothy grass (*Phleum pratense*), Sweet Vernal grass (*Anthoxanthum odoratum*), Perennial ryegrass (*Lolium perenne*), Kentucky Bluegrass (*Poa pratensis*)] (all from Stallergenes, France).

Statistical analysis

Statistical analyses were performed using R version 2.12.0 (The R Foundation for Statistical Computing, Vienna, Austria). To address the effect of allergic status on the risk of clinical *P.*

falciparum episodes, we performed Generalized Linear Mixed Models (GLMM) extended to pedigree data using the *pedigreemm* package for R to account for the non-independence of individuals because of family relationships, shared house and for repeated measures from the same individual (Technical Appendix). Correlated individual effects due to familial relationships were taken into account by using the pedigree-based genetic relatedness matrix that contains the genetic covariance among all pairs of individuals in the study cohort and is calculated using the pedigree information.[34] Shared house and repeated measures from the same individual were modelled as random effects. All random effects were assumed to be normally distributed, and conditional on these random effects, the dependent variable had: (i) a Binomial distribution when the studied phenotype was the occurrence of a clinical *P. falciparum* episode treated with anti-malarial therapy during a trimester, (ii) a Gaussian distribution when the studied phenotype was the logarithm of the maximum parasite density during a given clinical *P. falciparum* episode, and (iii) a Poisson distribution when the studied phenotype was the number of non-malaria episodes per trimester. The effects of allergy disease classes on these dependent variables were modelled as fixed effects. Allergy classes were reduced to two levels, *Severe* or *moderate vs*. *none* for analyses of asthma, atopic dermatitis and allergic rhinoconjunctivitis and *high probability vs*. *probable* and *unlikely* for atopic tendency. Co-variables included sickle cell trait[33], gender, number of days present on site during the trimester, trimestrial incidence of *P. falciparum* and age. Age was initially analysed as a continuous covariate. To assess the age-specific effect of allergy, age was categorised into two levels (<3.5 years of age and ≥3.5 years of age, based on the age of peak clinical incidence) and allergy class was nested within age class. The age threshold was varied from 1.5 years to 5.5 years of age and the data re-analysed to assess at which age there was the strongest effect. The association of allergy classes with IgE levels was analysed by box-cox transforming the data and fitting a GLMM with a normal distribution.

Results

Of the 205 eligible children aged under 15 years involved in the family-based longitudinal study, 175 (85.4 %) participated in the cross-sectional survey to assess the prevalence of related symptoms of allergic diseases. All eligible children present at the time of the survey

were included; no explicit refusal to participate was recorded. The study cohort was aged from 1 month to 14 years 11 months. The sex-ratio (male/female) was 0.94.

From 1994 until 2008, 143 of the children participating in the cross-sectional survey were present for at least 31 days in any trimester during the study period generating a total of 3,093 person-trimesters of presence (Supplementary Table S1). There were 2,065 treated *P. falciparum* clinical episodes (per individual: median 11, range 0-47)(Supplementary Table S2). The age peak of incidence of *P. falciparum* episodes occurred at 3 to 4 years of age (Figure 1). There were 1,868 non-malaria episodes (median 12, range 0-37) (Table S2). These non-malaria clinical presentations were associated with headache (38 %), chills (32 %), cough (13 %), vomiting (11 %) and diarrhoea (6 %).

The prevalence of moderate or severe asthma symptoms was respectively 2.3 % and 10.3 % (Table 1). The prevalence of moderate or severe allergic rhinoconjunctivitis symptoms was respectively 6.3 % and 10.3 %. The prevalence of moderate or severe atopic dermatitis symptoms was respectively 6.3 % and 2.9 %. On the basis of symptom severity, an atopic tendency was estimated to be unlikely for 68.0 %, probable for 9.1 % and highly probable for 22.9 % of the 175 children. The frequency of each allergy class in children for whom malaria data were available is shown in Table S1.

The risk of treated clinical *P. falciparum* infections was higher for children with high probability of atopy (OR 1.65, 95% confidence intervals 1.20 to 2.26; P=0.002) (Table 2), after adjusting for age, sickle cell trait and the exposure level. Gender was not found to be significant. Analysing the impact of atopy in children younger and older than the peak age of clinical incidence (3 to 4 years old), revealed that atopy increased the risk of *P. falciparum* episodes in children at an age greater than 3.5 years (OR 2.02, 1.39 to 2.93; $P=2x10^{-4}$), but not in children of age prior to the peak clinical incidence (OR 1.38, 0.92 to 2.08; P=0.124) (Table 2). This increased risk resulted in an ever increasing cumulative number of *P. falciparum* episodes with age beyond that of peak clinical incidence (Figure 2. See supplementary Figure S2 for model predictions for comparison).

Analysis by allergy category revealed that asthma (severe or moderate) increases the risk of *P. falciparum* episodes (OR 2.12, 1.46 to 3.08; $P= 8 x10^{-5}$) and this again only in children of age greater than 3.5 years old (OR 2.33, 1.50 to 3.61; $P= 1.5 x10^{-4}$). Atopic dermatitis increased the risk of clinical malaria in children older (OR 3.15, 1.56 to 6.33; $P= 1.3 x10^{-3}$) but

not younger than 3.5 years of age (Table 2). Allergic rhinoconjunctivitis was not associated with increased risk of clinical malaria at any age (Table 2). The impact of atopy, asthma and atopic dermatitis can be clearly seen in the ever-increasing number of cumulative *P. falciparum* episodes beyond the age of the onset of clinical immunity in the population, 3.5 years of age (Figure 2). There is no difference in the number of clinical malaria episodes prior to this age in individuals with or without an allergic condition. Analysis using different age thresholds (from 1.5 to 5.5 years of age) revealed similar OR for thresholds of 2.5, 3.5 and 4.5 years of age. The maximum OR for increased malaria occurred in children older than 4.5 years of age and with atopy or atopic dermatitis, whereas for the asthma group it occurred in children after 3.5 years of age (Supplementary Table S3).

There was no impact of any allergic disease on the number of non-malaria episodes by trimester (Supplementary Table S4).

The impact of atopy, asthma and atopic dermatitis on the maximum *P. falciparum* parasite density during a given clinical malaria episode mirrored that of the risk of *P. falciparum* episodes. Parasite density was significantly higher for children with allergic disease older than 3.5 years of age (Table 3 and supplementary Figure S3 for residuals of the fitted model). As the log-transformed data were left skewed, we additionally analysed using box-cox transformation and probit normalization of the data. The results were qualitatively the same (Supplementary text and Figures S4-S8). Allergic rhinoconjunctivitis had no impact on the parasite density (Table 3). Analysis using different age thresholds yielded similar qualitative conclusions as seen with the number of clinical episodes (Table S3).

Individuals with moderate or severe symptoms of atopic dermatitis had significantly higher specific IgE titres against *Ae. aegypti* (P=0.004) and *An. gambiae* SGE (P<0.001). There were no detectable specific anti-*P. falciparum* IgE. Individuals with moderate or severe symptoms of allergic rhinoconjunctivitis did not have significantly higher IgE titres against the tested graminae (P=0.28), although titres decreased with age (P=0.035). There was also no effect of asthma on IgE titres against the house dust mite spp. tested (*D. farinae* P=0.60 & *D. pteronyssinus* P=0.27).

Only five individuals were infested with helminths (two *Ancylostoma*, one *Strongyloides*, one *Trichuris* and one *Enterobius*).

Discussion

Principal findings

Establishing the allergic status of children up to the age of 15 years old followed for malaria since birth, revealed an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

Strengths and weaknesses of the study

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association. In addition, although allergy diagnosis for children under 2 years of age is not considered reliable, there were only 15 individuals under 2 at the time of the allergy study of the 143 for whom malaria and allergy data were available.

Meaning of the study

Under intense malaria transmission, after repeated exposure to the parasite, children develop a clinical immunity[35], whereby they tolerate elevated parasite densities without showing clinical symptoms. In this cohort, the population mean onset of clinical immunity occurred at 3 to 4 years of age. Although clinical immunity is accompanied by a reduction in parasite density, effective anti-parasite immunity develops much more slowly[36] with individuals achieving a state of premunition, whereby they maintain low-grade parasite densities in an asymptomatic state.[37] We show here that children with clinically defined asthma or atopic dermatitis have an increased risk of presenting with *P. falciparum* malaria episodes requiring treatment once passing the age of peak clinical incidence. They also had higher parasite density during clinical episodes, suggesting a reduced ability to control parasite replication. The observed increase in clinical incidence of malaria in patients with asthma or atopic dermatitis is not likely to be the result of increased frailty of such individuals; these individuals did not come more frequently to the clinic with non-malaria

symptoms. Our previous genome linkage study identifying chromosomal regions[20] associated with malaria that overlap with those previously shown to be linked to asthma/atopy suggests that there may be a shared genetic basis to these pathologies rather than any causative effect of one on the other. This is consistent with the increased susceptibility to malaria of mouse atopic models.[23]

Comparison with other studies

A previous study in Ethiopia (East Africa) found that a history of malaria (yes/no) increased risk of atopic dermatitis in 306 cases compared to 426 controls as characterized using the ISAAC questionnaire.[22] The only other epidemiological study that has previously examined the link between malaria and atopy[38] also interpreted the result from the perspective of the impact of malaria on atopy. They examined the re-infection rate with *P. falciparum* over a 5-year period in 91 children that were subsequently classified as atopic or not using skin prick tests (SPT) with house dust mite antigen. Their conclusion was that, as with measles[13] and tuberculosis[15], malaria infection reduces atopy. However, the study lacked previous infection data since birth of the participating individuals and focussed on atopy as determined by SPT against a single allergen. The case-control study of atopic dermatitis risk factors cited above found no overall association between allergen skin sensitization and atopic dermatitis. We also found no evidence of increased IgE titres against house dust mites in the asthmatic or atopic dermatitis groups or against grass pollen in individuals with allergic rhinoconjunctivitis. Such differences likely reflect the different IgE reactivity profiles due to differences in allergen exposure in Africa.[39] There was no evidence of anti-parasite IgE in this cohort of children. We previously showed that circulating anti-parasite IgE titres were strongly positively correlated with anti-mosquito saliva IgE, but became undetectable following malaria exposure, potentially being bound to effector cells.[33] Only mosquito saliva, a known major local allergen, induced a specific IgE response at significantly higher titres in individuals with atopic dermatitis.

Although the immune effectors of clinical immunity are still poorly defined, there is strong evidence that acquired anti-parasite immunity is IgG-dependent[8] and cytophilic immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by opsonisation and/or by Antibody Dependent Cellular Immunity play an important role in premunition.[37] The higher parasite density during symptomatic episodes observed in the

asthma group suggests impaired development of acquired immunity. Impaired acquisition of immunity to malaria in children with asthma or atopic dermatitis may stem from their imbalanced Th1/Th2 response. Indeed, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*. Dendritic cells that are oriented to a Th2 phenotype are more susceptible to orient the acquired immune response towards a Th2 profile.[40] Orientation of the immune response towards a Th2 profile by asthma or atopic dermatitis would result in a poor Th1 response (and hence development of protective IgG immunoglobulins), considered to be the dominant arm of the immune response enabling resistance to infectious disease in children.[41]

Many studies have revealed an important role of histamine, a key downstream effector molecule in allergic reaction, in the outcome of a malaria parasite infection.[24-26,42-45] Moreover, reports indicate that components of the innate immune system, including eosinophils, basophils, and mast cells (MCs), could play important roles in the pathogenesis of malaria.[42] Increased levels of histamine in plasma and tissue, derived from basophils and MCs, notably following stimulation by IgE through the high affinity receptor FcεR1, are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[25,26] Chlorpheniramine, a HR1agonist reversed resistance to chloroquine and amodiaquine both *in vivo* and *in vitro*.[43] Moreover, astemizole, another HR1 agonist, was identified as an anti-malarial agent in a clinical drug library screen.[44] Finally, *P. falciparum* produces translationally controlled tumor protein, which is a homolog of the mammalian histamine-releasing factor that causes histamine release from human basophils.[45]

Further research

Our results provide the first birth cohort study addressing the link between malaria and allergic diseases. They contribute to a growing body of evidence that the pathologies are related. ISAAC has revealed a steady but significant increase in prevalence rates of asthma and allergic diseases in Africa. Whilst the majority of studies have focused on large cities, there is increasing urbanization throughout Africa, as well as improved access to primary health care in many areas. A key concern for ISAAC is the extent to which such societal evolution will result in an increase in allergic diseases. Increased urbanization in sub-Saharan Africa is changing the epidemiology of malaria and although resulting in a decrease in risk, will result in more severe clinical malaria in older individuals.[46,47] Moreover, a large

consumption of anti-malarial drugs in the urban areas provides substantial drug pressure fostering, the selection of drug-resistant parasites. Despite the encouraging recent decrease in malaria incidence rates, even in rural areas, an additional significant concern is the extent to which such an increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines,[48] administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials[49] and alleviate the non-infectious consequences of atopy. Clinical intervention studies should be envisaged.

What is already known on this topic

There are several reports of the beneficial effects of anti-histamines for malaria chemoprophylaxis[24-26,48] as well as our previous work[20] showing that chromosomal regions associated with malaria are also linked to allergy and atopy.[17-19] There are two epidemiological studies showing opposite effects of malaria on atopy.[22,38]

What this study adds

Using a longitudinal malaria study birth cohort, we identified an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred only after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: The allergy study was approved by the Senegalese National Ethics committee (2009/N°46). Renewed approval of the longitudinal malaria study was obtained from the same committee (2006/N°969).

Data sharing: The allergy database will be made available on-line. The longitudinal malaria data set will be made available following discussion with the coordinators of the three Institutes that govern the dataset through contact with the corresponding author.

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Table 1 Classification of Asthma, Allergic rhinoconjunctivitis, Atopic dermatitis and overall Atopic status according to ISAAC questionnaire in children aged 0-14 from a malaria birth cohort. N is total number of children examined and n-malaria represents those for whom malaria data were recorded. F is the number of females and M the number of males.

	N (F/M)	%	n-malaria (F/M)
Asthma symptoms			
None	153 (73/80)	87.43	125 (59/66)
Moderate	4 (1/3)	2.29	4 (1/3)
Severe	18 (6/12)	10.29	14 4/10)
Rhinoconjunctivitis symptoms			
None	146 (64/82)	83.43	120 (52/68)
Moderate	11 (8/3)	6.29	9(6/3)
Severe	18 (6/12)	10.29	14 (6/8)
Atopic dermatitis symptoms			
None	159 (75/84)	90.86	128 (60/68)
Moderate	11 (1/10)	6.29	11 (1/10)
Severe	5 (4/1)	2.86	4 (3/1)
Atopic tendency			
Unlikely	119 (56/63)	68.00	97 (46/51)
Probable	16 (8/8)	9.14	14 (6/8)
Highly probable	40 (16/24)	22.86	32 (12/20)

Table 2 Impact of allergy status on risk of *P. falciparum* clinical episodes. Shown are the *P* values and adjusted Odds Ratios with 95% confidence intervals calculated from the mixed model analyses. Values for the covariables Age (≥3.5 years of age compared to <3.5 years of age), Trimestrial incidence of *P. falciparum* clinical episodes and HbAS (beta-globin sickle cell trait; AS compared to AA) are those from the Asthma model analysis. For clarity significant co-variables are shown in bold.

	Age groups <3.5 years>	ORa	95% Confidence Intervals		*P* value
			Lower	Upper	
Atopy	**Both**	**1.65**	**1.20**	**2.26**	**2.0×10^{-3}**
	<3.5	1.38	0.92	2.08	0.124
	≥3.5	**2.02**	**1.39**	**2.93**	**2.1×10^{-4}**
Asthma	**Both**	**2.12**	**1.46**	**3.08**	**8.0×10^{-5}**
	<3.5	1.50	0.90	2.50	0.122
	≥3.5	**2.33**	**1.50**	**3.61**	**1.5×10^{-4}**
Atopic dermatitis	Both	1.05	0.65	1.70	0.842
	<3.5	0.84	0.49	1.46	0.539
	≥3.5	**3.15**	**1.56**	**6.33**	**1.3×10^{-3}**
Rhinoconjunctivitis	Both	0.96	0.65	1.41	0.818
	<3.5	1.05	0.64	1.72	0.853
	≥3.5	0.95	0.60	1.52	0.834
Age ≥3.5		**0.48**	**0.40**	**0.57**	**2.7×10^{-15}**
Trimestrial incidence		**1.01**	**1.00**	**1.01**	**1.8×10^{-6}**
HbAS		**0.24**	**0.12**	**0.47**	**3.7×10^{-5}**

Table 3 Impact of allergy status on the maximum *P. falciparum* parasite density during a clinical malaria episode. Shown are the back-transformed mean parasite densities per microlitre and standard errors (SEM) estimated from the GLMM analyses after taking into account the other co-variables. Significantly different effects are shown in bold for clarity.

Allergic condition	Age groups	Allergic status (No/Yes)	Mean parasite density	SEM	*P* value
Atopy	**Both**	**N**	**76.3**	13.8	
		Y	**131.0**	36.4	**0.0158**
	<3.5	N	114.3	23.7	
		Y	171.1	56.0	0.148
	≥3.5	**N**	**48.4**	9.8	
		Y	**114.8**	37.1	**$9.5x10^{-4}$**
Asthma	**Both**	**N**	**78.1**	14.4	
		Y	**148.5**	44.3	**$3.8\ x10^{-3}$**
	<3.5	N	114.8	24.3	
		Y	171.9	74.5	0.167
	≥3.5	**N**	**51.3**	9.7	
		Y	**105.3**	41.0	**$6.2\ x10^{-3}$**
Atopic dermatitis	Both	N	82.6	15.0	
		Y	93.9	38.9	0.605
	<3.5	N	122.6	25.5	
		Y	133.9	63.5	0.425
	≥3.5	**N**	**52.3**	11.0	
		Y	**135.4**	70.7	**0.014**
Rhinoconjunctivitis	Both	N	81.5	14.8	
		Y	111.4	39.0	0.570
	<3.5	N	118.8	25.1	
		Y	166.3	69.9	0.537
	≥3.5	N	54.6	11.3	
		Y	80.9	33.7	0.327

Figure 1 Annual incidence rate of clinical *P. falciparum* episodes per 100 children (bold line). In order to overcome the fluctuations of the annual incidence rate, we fit second (dotted line), third (dashed line) and fourth (solid line) degree polynomial trend lines to the data (bold line). The corresponding R-squared values are 0.70, 0.91 and 0·99 respectively indicating an accurate fit for third and fourth order polynomials. The inflexion on these two trend lines indicates the onset of acquisition of clinical immunity at approximately 3 to 4 years of age.

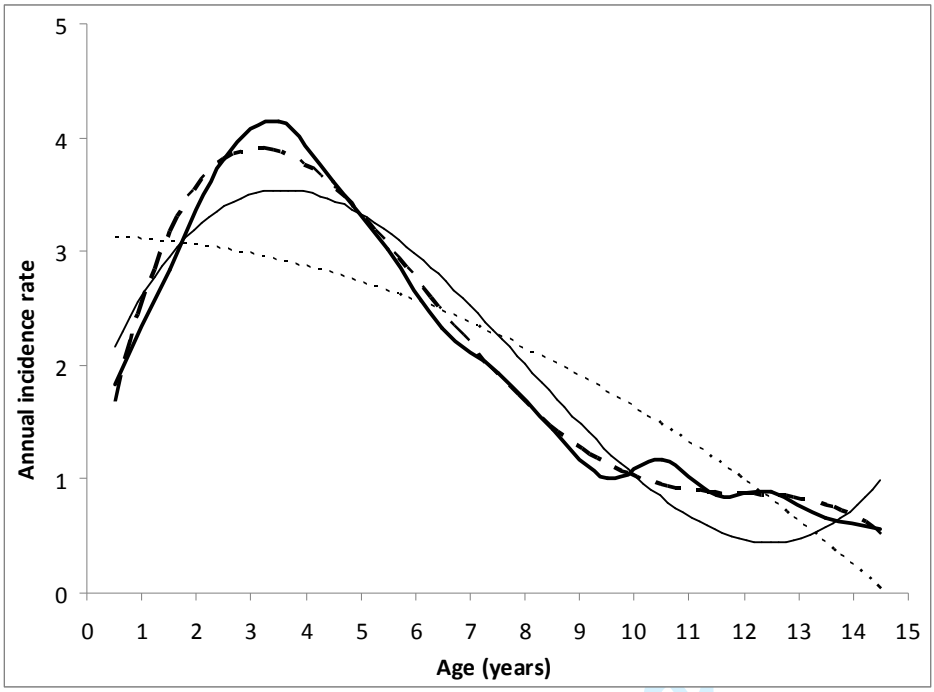
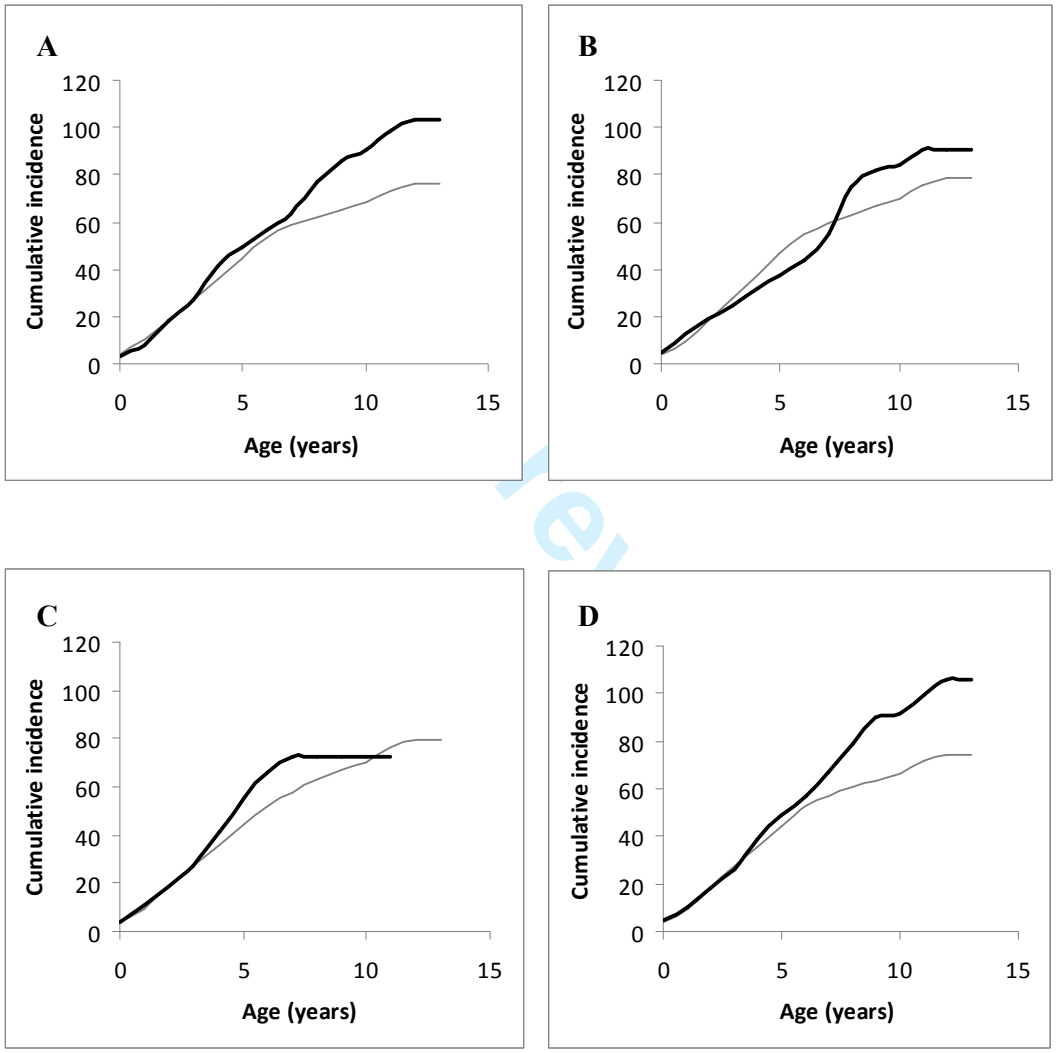


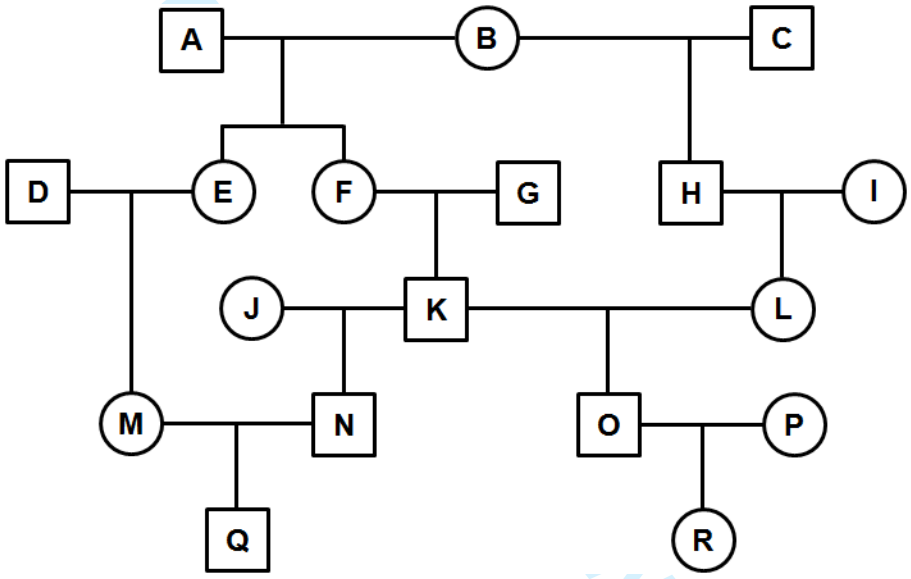
Figure 2 Mean cumulative number of *P. falciparum* clinical episodes with age for the (A) Asthma, (B) Rhinoconjunctivitis and (C) Atopic dermatitis classes and overall Atopy class (D) (bold lines) compared to individuals without symptoms of each respective allergy type (thin lines). In all cases moderate and severe classes are combined and compared to individuals without allergy symptoms. Note there are no children older than 11 years of age with Atopic dermatitis.



Pedigree-based genetic relatedness

The Genetic covariance between two individuals can be computed using the pedigree information. For individuals A and B, a given pair in a pedigree, the genetic covariance is computed as $r(A,B) = 2\times coancestry(A,B)$ where the *coancestry* between A and B is calculated referring to the method presented by Falconer and Mackay in 1996 (Falconer and Mackay 1996): $coancestry(A,B) = \Sigma_p(1/2)^{n(p)}\times(1 + I_{Common\ Ancestor})$ where p is the number of paths in the pedigree linking A and B, $n(p)$ the number of individuals (including A and B) for each path p and I_X is the *inbreeding* coefficient of X also equal to the *coancestry* between the two parents of X, I_X is set to 0 if X is a founder.

Illustration: Consider, as an example, the pedigree below containing 18 individuals named {A, B, …, R} for the calculation of genetic covariance's.



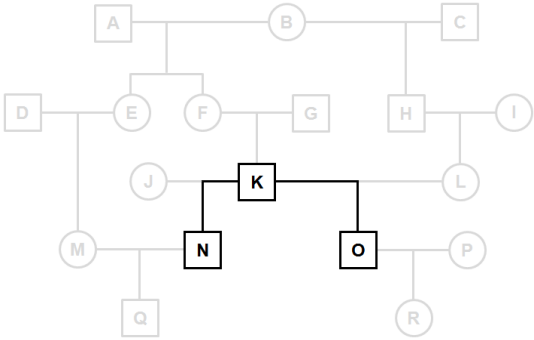
Pedigree structure.

The genetic relatedness between individuals N and O is equal to 0.266. This value is calculated as followed:
The number of paths linking N and O from the pedigree structure above is $p = 2$.
As illustrated below:

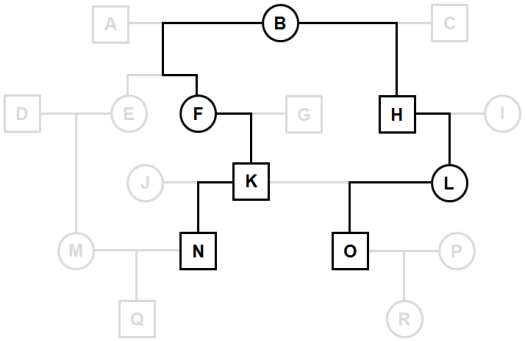
- **Path 1** contains $n(1) = 3$ individuals {N, K, O} with K as the common ancestor. Inbreeding coefficient of K, I_K , is the *coancestry* between the two parents of K (F and G) and is null because F and G are not genetically linked.
- **Path 2** contains $n(2) = 7$ individuals {N, K, F, B, H, L, O} with B as the common ancestor. Inbreeding coefficient of B, I_B , is null because B is a founder.

Therefore, genetic relatedness between individuals N and O is:

$$= 2\times(0.5^{n(1)}\times(1+I_K) + 0.5^{n(2)}\times(1+ I_B))$$
$$= 2\times(0.5^{3}\times(1+0) + 0.5^{7}\times(1+0)) = 0.266$$



Path 1 linking N and O.



Path 2 linking N and O.

Defining an equivalent model design where individual effects are independent using the genetic relatedness matrix:

Let us rename $Y^* = l(\mu)$. Y^* can be consider as a linearization of the phenotype through the link function l. The expected mean of Y^* and the variance of Y^* are:

(i) $E(Y^*) = E(X\beta + Z\gamma + \varepsilon)$
$= E(X\beta) + E(Z\gamma) + E(\varepsilon) = X \times E(\beta) + Z \times E(\gamma) + E(\varepsilon)$
$= X\beta$ (asymptomatically).

(ii) $Var(Y^*) = Var(X\beta + Z\gamma + \varepsilon)$
$= Var(Z\gamma + \varepsilon)$ (as $X\beta$ is the fixed part, thus has variance equal to 0)
$= Var(Z\gamma) + Var(\varepsilon)$ (as γ and ε are independent)
$= Z \times Var(\gamma) \times Z^T + Var(\varepsilon)$ (Z^T is the transpose of Z)
$= Z(A\sigma_g^2)Z^T + I\sigma_r^2$
$= ZAZ^T\sigma_g^2 + I\sigma_r^2$

If individuals were independent, i.e. $A = I_N$, variance of Y^* could be expressed as $ZZ^T\sigma_g^2 + I\sigma_r^2$. However, using linear algebra theory by the method "Cholesky decomposition of a matrix", we can show that there is an equivalent expression of the variance of Y^* corresponding to the modeling of data from independent individuals, having γ^* as an equivalent vector of random effects and Z^* an equivalent design matrix relating γ^* to Y^* so that:
$Var(Y^*) = Z^*(I\sigma_g^2)Z^{*T} + I\sigma_r^2$. $I\sigma_g^2$ is then the covariance matrix of the equivalent independent random individual effects γ^*.

Theorem: *Cholesky decomposition of a matrix*
If A is a symmetric positive-definite matrix, there is a triangular matrix L so that A can be written as $A = LL^T$. L can be seen as the "square root" of the matrix A.
Note that the genetic relatedness matrix A computed using the pedigree information (Falconer and Mackay 1996) is a positive-definite matrix, unless identical twins are in the pedigree in which case it would be positive semi-definite.

Equivalent model with independent random effects: We set $A = LL^T$ then:
$Var(Y^*) = Z(A\sigma_g^2)Z^T + I\sigma_r^2$
$= Z(LL^T\sigma_g^2)Z^T + I\sigma_r^2$

$= ZLL^{T}Z^{T}\sigma_g^2 + I\sigma_r^2$
$= (ZL)(ZL)^{T}\sigma_g^2 + I\sigma_r^2$
$= (Z^*)(Z^*)^{T}\sigma_g^2 + I\sigma_r^2$ (where we set $Z^* = ZL$)

Then, if we define $\gamma^* = L^{-1}\gamma$, we can rewrite the model as:

$Y^* = X\beta + Z^*\gamma^* + \varepsilon$ (because $Z\gamma = Z(LL^{-1})\gamma = (ZL)(L^{-1}\gamma) = Z^*\gamma^*$),

and the γ_i^* are independent, in other terms $Var(\gamma^*) = I\sigma_g^2$, as demonstrated below:

We assumed that $\gamma \sim N(0, A\sigma_g^2)$. Then $\gamma^* = L^{-1}\gamma$ is also distributed as a multivariate Normal with mean $E(\gamma^*) = L^{-1}E(\gamma) = L^{-1}\times 0 = 0$ and variance:

$Var(\gamma^*) = (L^{-1})\times Var(\gamma)\times(L^{-1})^{T}$
$= (L^{-1})\times A\sigma_g^2\times(L^{-1})^{T} = (L^{-1})LL^{T}(L^{-1})^{T}\sigma_g^2$
$= (L^{-1}L)(L^{-1}L)^{T}\sigma_g^2$
$= I\sigma_g^2$

The random effects are now independent and then the classical mixed model assuming independence between levels (here individuals) is applied, and the estimate of fixed effects obtained are fine, i.e. corrected for genetic relationships.

References

Falconer DS, Mackay TFC (1996) Introduction to Quantitative Genetics. 4th Edn. London: Longman.

Supplementary Tables

Table S1 Number of person-trimesters contributed by number of children by age class and the number who had severe/moderate allergy symptoms, for whom malaria data were also available. AS – Asthma, AD – Atopic dermatitis, RC – Rhinoconjunctivitis. Shown also are the numbers of these individuals suffering from two or all three allergy conditions.

Age group	N° person-trimesters	N° people	AS	AD	RC	AS+AD	AS+RC	AD+RC	AS+AD+RC
]1	7	6	1	2	2	0	1	0	0
]2	21	9	0	1	3	0	0	0	0
]3	48	11	1	1	2	0	0	1	0
]4	119	12	1	2	3	0	0	1	0
]5	102	11	3	4	3	2	1	2	1
]6	125	11	1	1	0	0	0	0	0
]7	303	11	1	2	1	1	0	0	0
]8	340	12	1	1	1	1	0	0	0
]9	362	10	2	0	1	0	1	0	0
]10	610	17	1	0	3	0	0	0	0
]11	77	4	2	1	0	0	0	0	0
]12	484	16	3	0	3	0	1	0	0
]13	390	10	1	0	0	0	0	0	0
]14	105	3	0	0	1	0	0	0	0
Total	3093	143	18	15	23	4	4	4	1

Table S2 Summary of total number of person-trimesters with non-malaria and symptomatic *P. falciparum* clinical presentations and total number of non-malaria episodes according to age class. Given are the number of people contributing to each type of presentation.

	Age group (years)	
	<3·5	**≥3·5**
Total person-trimesters	1283	1810
People	126	113
Total *P. falciparum* symptomatic trimesters	963	1102
People	114	108
Total non-malaria episodes	754	1114
People	123	109

Table S3 Effect of changing age threshold on impact of allergy on the risk of clinical malaria and concomitant parasite density. Given are Odds Ratio with 95% confidence intervals, for clinical malaria episodes and the beta coefficient and standard error for parasite density. Corresponding P values are also given. Values are from the nested GLMM analyses.

A. Malaria episodes							**B. Parasite density**				
Age cut-off (years)	OR	95% CI	P value	OR	95% CI	P value	Age cut-off	beta coeff (se)	P value	beta coeff (se)	P value
Atopy		above threshold			below threshold		Atopy	above threshold		below threshold	
1.5	1.80	1.25-2.59	1.7x10-3	1.57	0.85-2.89	0.15	1.5	0.70 (0.27)	9.2x10-3	0.54 (0.35)	0.12
2.5	2.00	1.39-2.88	2.0x10-4	1.23	0.76-1.99	0.40	2.5	0.79 (0.26)	2.6x10-3	0.35 (0.29)	0.23
3.5	2.02	1.39-2.93	2.1x10-4	1.38	0.92-2.08	0.12	3.5	0.85 (0.26)	9.5x10-4	0.37 (0.26)	0.15
4.5	2.10	1.42-3.10	1.6x10-4	1.41	0.98-2.04	0.063	4.5	0.87 (0.25)	6.9x10-4	0.40 (0.23)	0.09
5.5	1.64	1.07-2.52	0.02	1.67	1.17-2.37	0.004	5.5	0.73 (0.27)	7.4x10-3	0.48 (0.22)	3.4x10-3
Asthma							Asthma				
1.5	1.98	1.29-3.03	1.8x10-3	1.46	0.69-3.19	0.34	1.5	0.66 (0.31)	0.03	0.30 (0.44)	0.48
2.5	2.30	1.49-3.55	1.6x10-4	1.15	0.63-2.09	0.65	2.5	0.78 (0.30)	0.01	0.26 (0.36)	0.48
3.5	2.33	1.50-3.61	1.5x10-4	1.50	0.90-2.50	0.12	3.5	0.82 (0.30)	6.2x10-3	0.43 (0.31)	0.17
4.5	2.30	1.48-3.59	2.4x10-4	1.76	1.11-2.80	0.017	4.5	0.81 (0.29)	5.8x10-3	0.56 (0.28)	0.049
5.5	1.98	1.22-3.22	0.006	2.06	1.33-3.18	0.0011	5.5	0.72 (0.31)	0.02	0.62 (0.27)	0.02
Atopic Dermatitis							Atopic Dermatitis				
1.5	2.05	1.18-3.56	0.01	0.91	0.42-1.97	0.80	1.5	0.80 (0.37)	0.03	0.72 (0.46)	0.12
2.5	2.49	1.36-4.57	3.1x10-3	0.82	0.44-1.53	0.53	2.5	0.77 (0.38)	0.044	0.52 (0.39)	0.19
3.5	3.15	1.56-6.33	1.3x10-3	0.84	0.49-1.46	0.54	3.5	0.99 (0.40)	0.014	0.28 (0.35)	0.42
4.5	3.79	1.61-8.92	2.3x10-3	0.94	0.57-1.57	0.82	4.5	0.98 (0.47)	0.036	0.29 (0.32)	0.37
5.5	1.33	0.47-3.77	0.59	1.19	0.73-1.96	0.49	5.5	0.26 (0.61)	0.67	0.38 (0.31)	0.22
Rhinoconjunctivitis							Rhinoconjunctivitis				
1.5	1.04	0.66-1.62	0.88	1.01	0.51-2.01	0.98	1.5	0.36 (0.32)	0.27	0.18 (0.41)	0.66
2.5	1.01	0.64-1.61	0.96	0.96	0.55-1.68	0.89	2.5	0.28 (0.33)	0.40	0.25 (0.35)	0.48
3.5	0.95	0.60-1.52	0.83	1.05	0.64-1.72	0.85	3.5	0.31 (0.32)	0.33	0.19 (0.31)	0.54
4.5	0.87	0.54-1.42	0.59	1.06	0.68-1.66	0.79	4.5	0.20 (0.32)	0.53	0.22 (0.28)	0.44
5.5	0.81	0.48-1.36	0.43	1.07	0.70-1.64	0.74	5.5	0.10 (0.33)	0.75	0.23 (0.27)	0.39

Table S4 Frequency of non-malaria episodes (number of days of presence divided by number of non-malaria episodes) according to allergic status and age group. The *P* value is that from the GLMM analyses of the effect of allergic status by age group on the number of non-malaria episodes per person-trimester.

Allergic condition	Allergic status (No/Yes)	Age group (years) <3·5	Age group (years) >3·5	*P* value
Atopy	N	78·2	85·9	0·105
	Y	87·2	102·6	
Asthma	N	79·6	87·3	0·319
	Y	82·5	100·2	
Atopic dermatitis	N	80·9	88·2	0·323
	Y	73·4	101·9	
Rhinoconjunctivitis	N	77·9	88·3	0·167
	Y	94·9	91·8	

Figure S1. Incidence of clinical cases per 100 person-trimesters in children under 15 years of age.

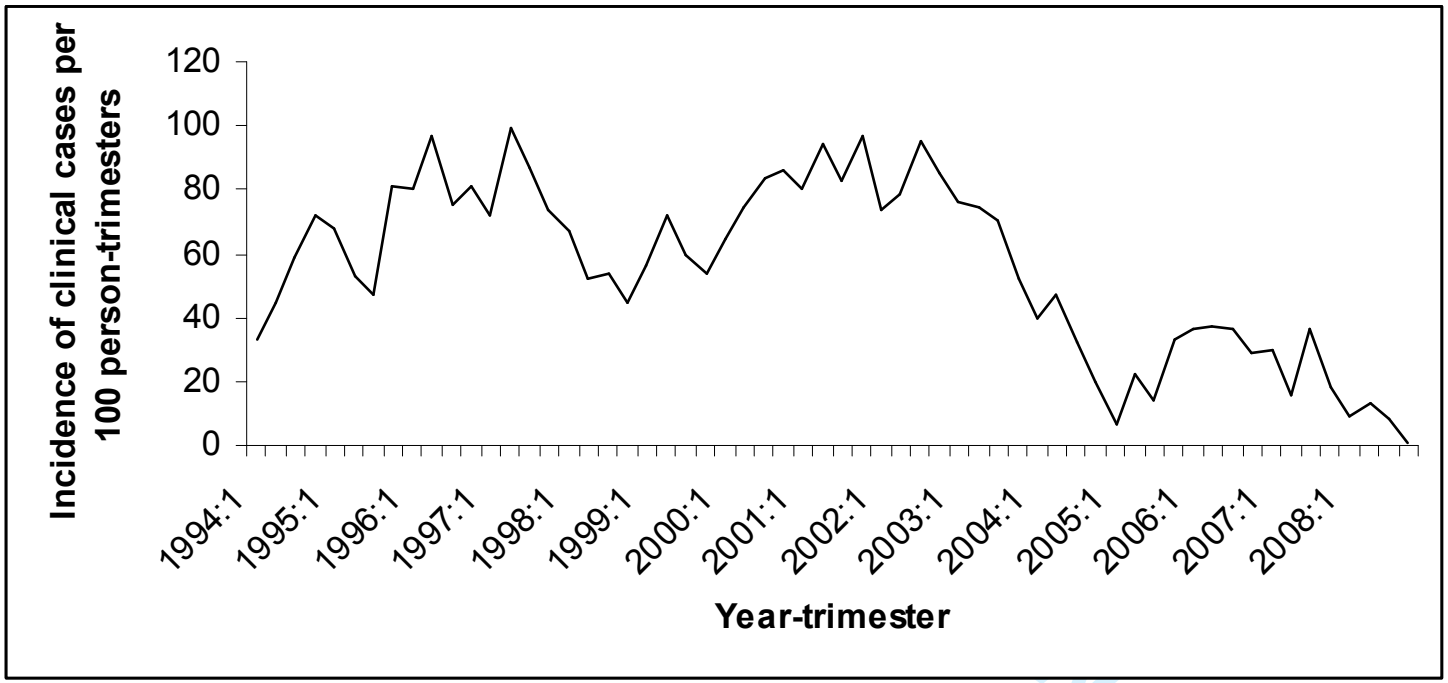


Figure S2. Cumulative incidence of clinical cases according to allergy class predicted by the statistical model.

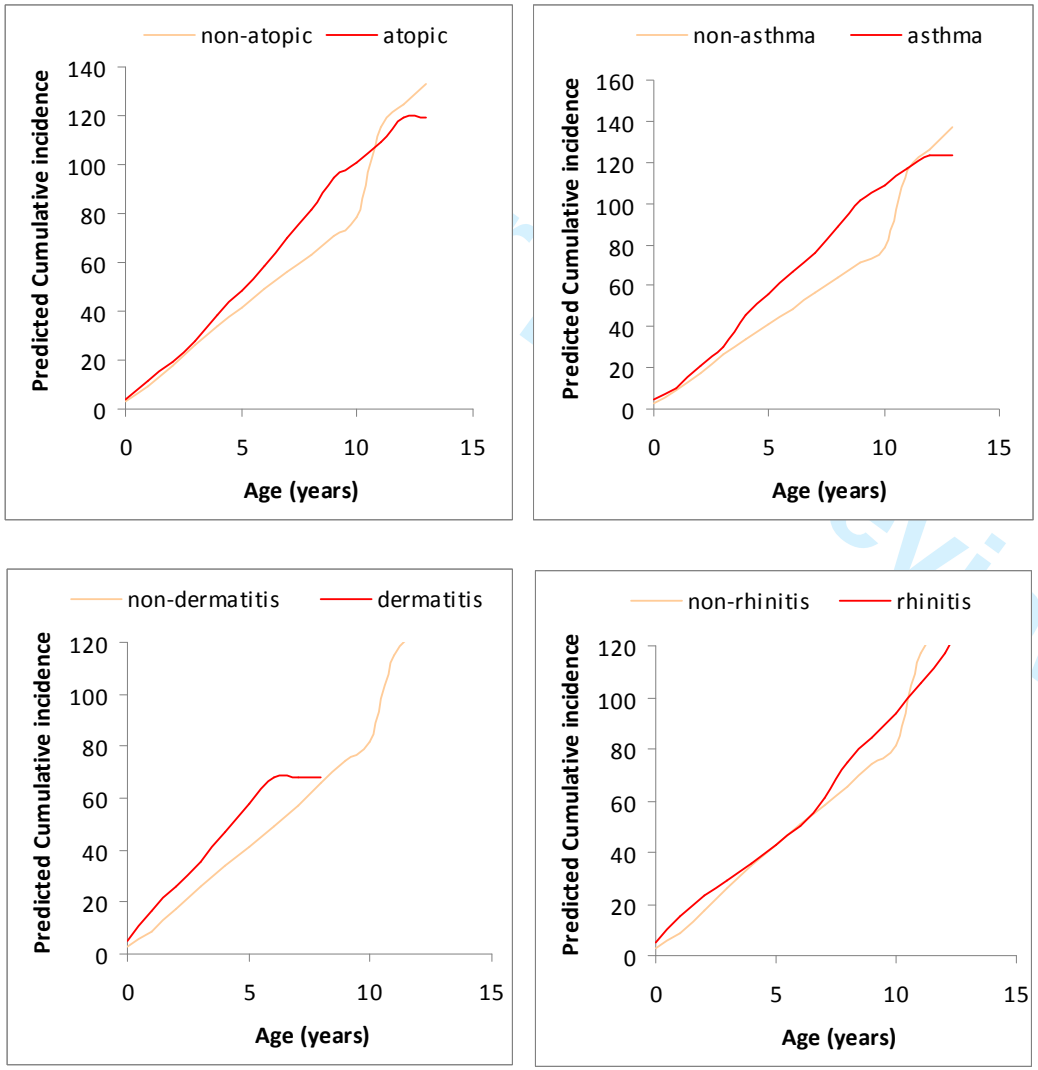
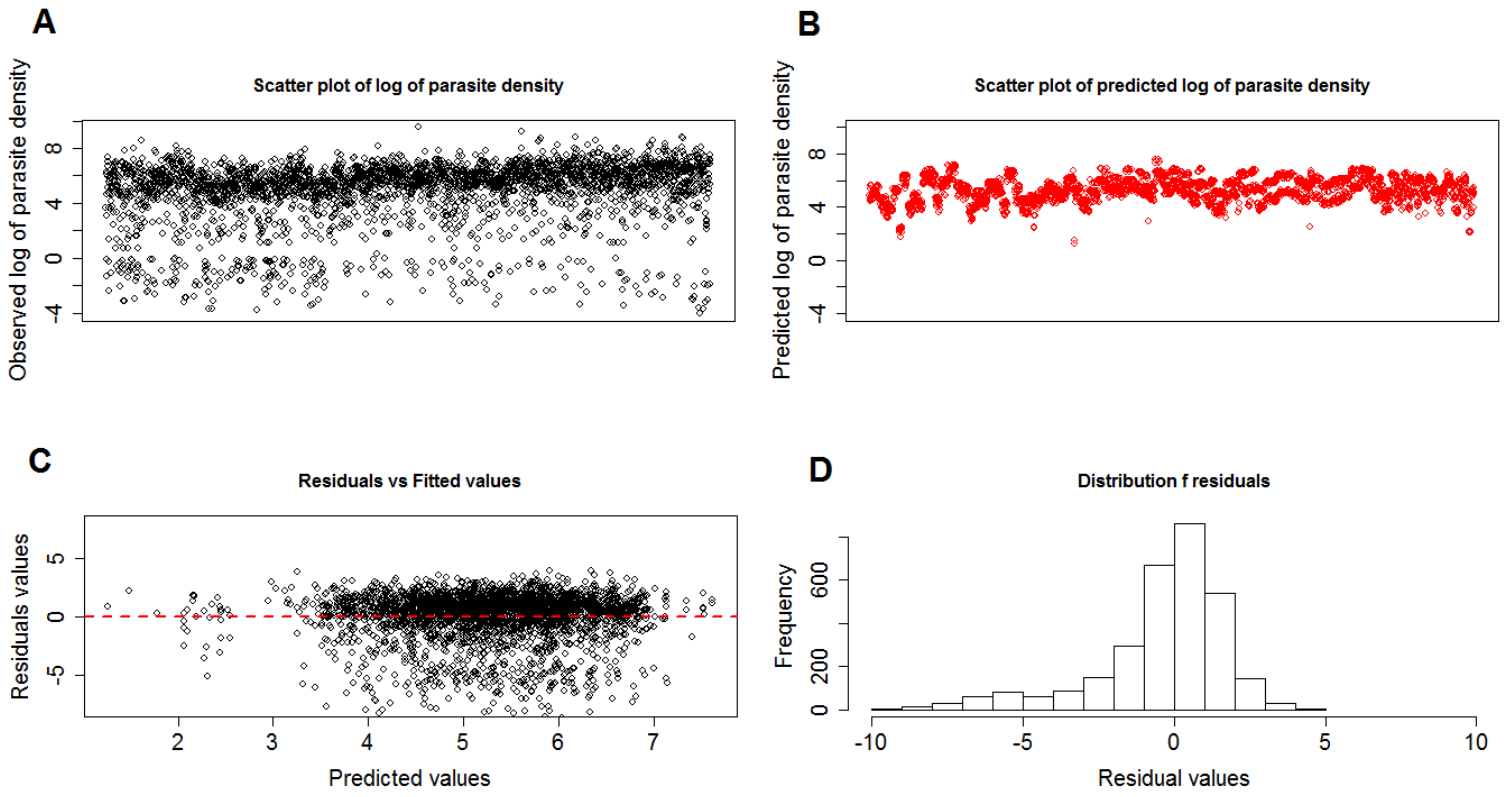


Figure S3. Graphical control model for parasite density

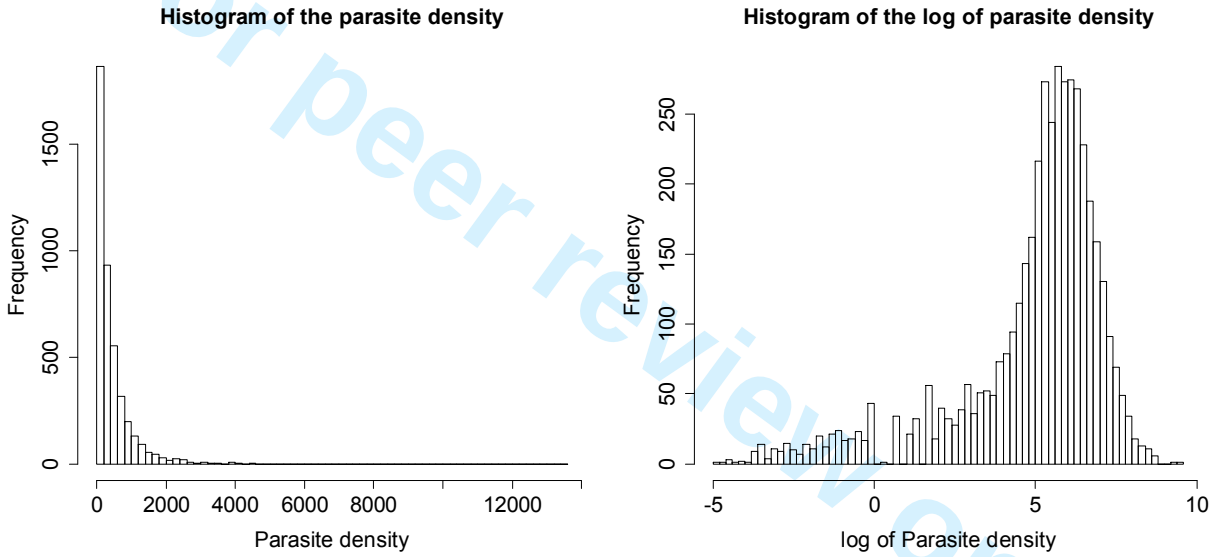
These figures provide a graphical checking of model goodness of fit. Figure A is the scatter plot of the natural logarithm of the observed parasite density and is compared to Figure B, which is the scatter plot of the natural logarithm of the predicted parasite density by the model; on both figures A and B the y-axes give the values for the log of the parasite density. Figure C shows the distribution of the residuals with the predicted values and Figure D is the histogram of the residuals; both figures C and D show the residuals normally distributed around zero.



Analysis using box-cox transformation and probit normalization

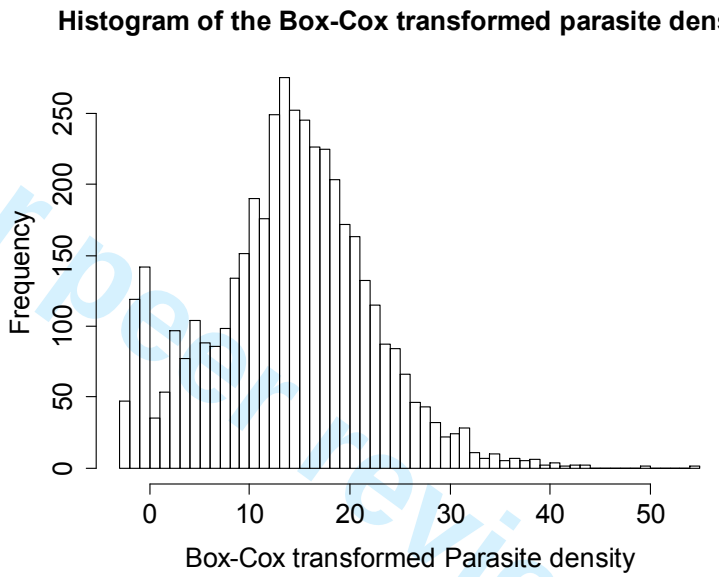
The model we fitted on the parasite density ("*pf_density*") has used as outcome variable the natural logarithm of *pf_density* (equivalent to a Box-Cox for which the parameter is null). As shown on Figure S4 the distribution of log(*pf_density*) is not perfectly normal, it is left-skewed.

Figure S4. Histogram of *pf_density* and log(*pf_density*)



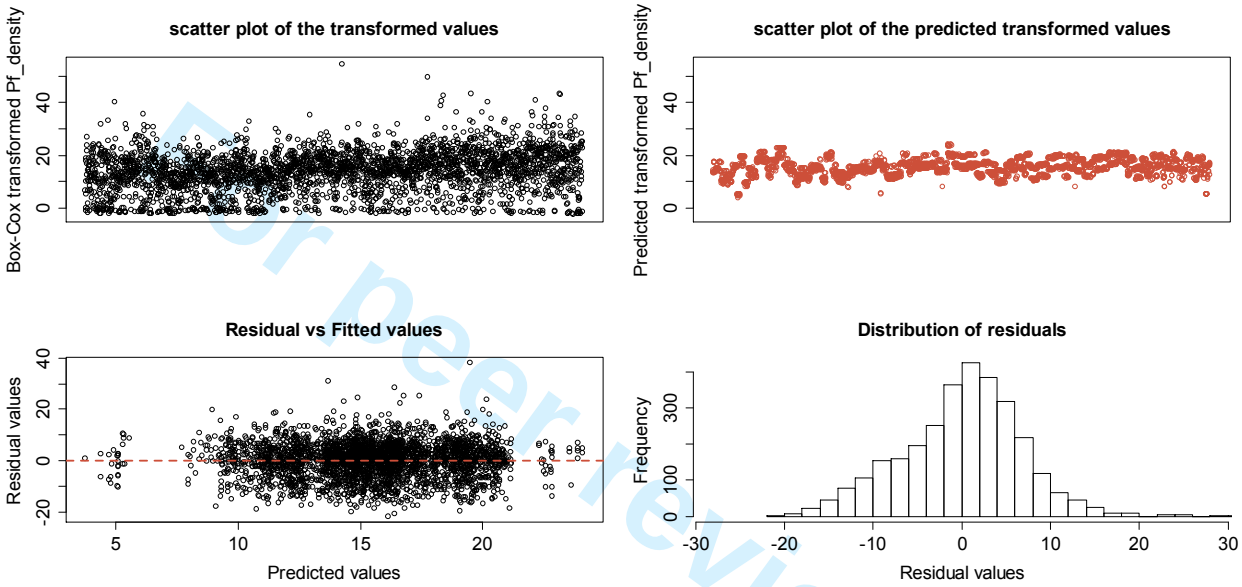
We add here the case for a Box-Cox transformation of the parasite density where the parameter is $\lambda = 0.3$, this parameter value was obtained as optimal using the R- function named "boxcox" from the "MASS" library. Then the Box-Cox transformation of the parasite density is $y = (pf\_density^{0.3} - 1)/0.3$ having the distribution shown on Figure S5 below.

Figure S5. Histogram of the Box-Cox transformation of pf_density using a λ parameter of 0.3



With this Box-Cox transformed parasitemia as outcome variable, our results are maintained. Note that this distribution is not "perfectly" normal. However, the corresponding graphical control of the model adequation presented on Figure S6 below shows residuals more close to the normal distribution than those for log(*pf_density*) as outcome.

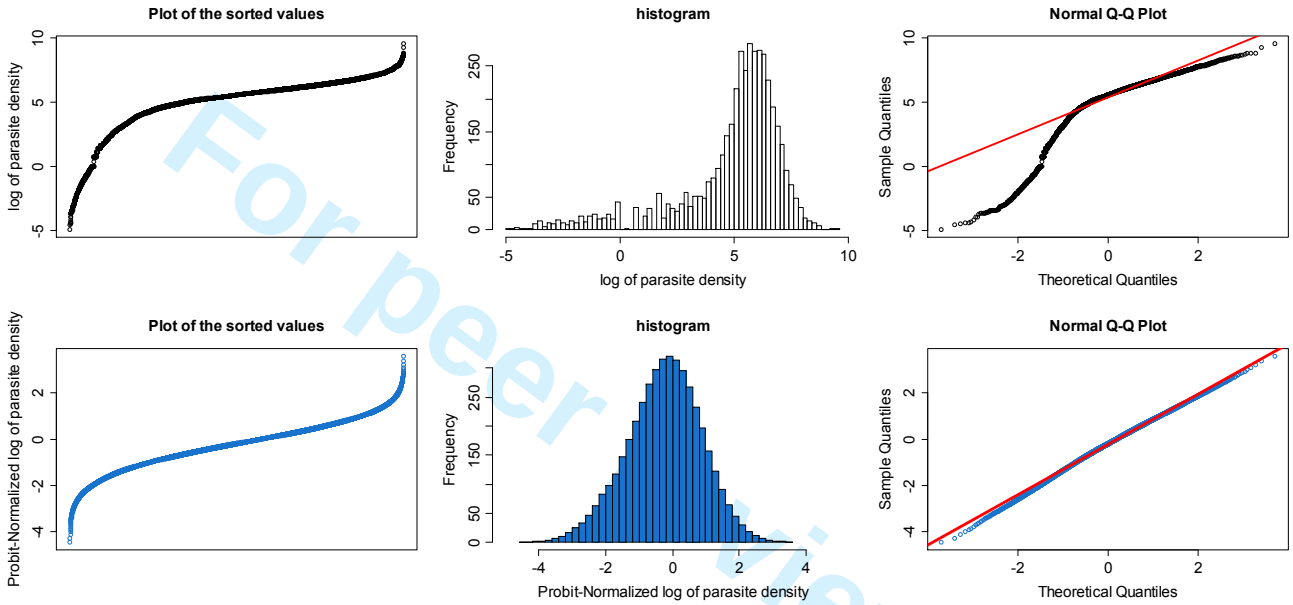
Figure S6. Graphical control of the model adequation for y = Box-Cox(*pf_density*, λ = 0.3)



Although using a mixed model approach based on an extreme value distribution would provide a more robust validation of these results, the method we used incorporating pedigree information was developed through an R-package known as "pedigreemm" that allows just for a limited number of distribution laws, which do not include extreme value distributions like the Gumbel or Weibull distributions.

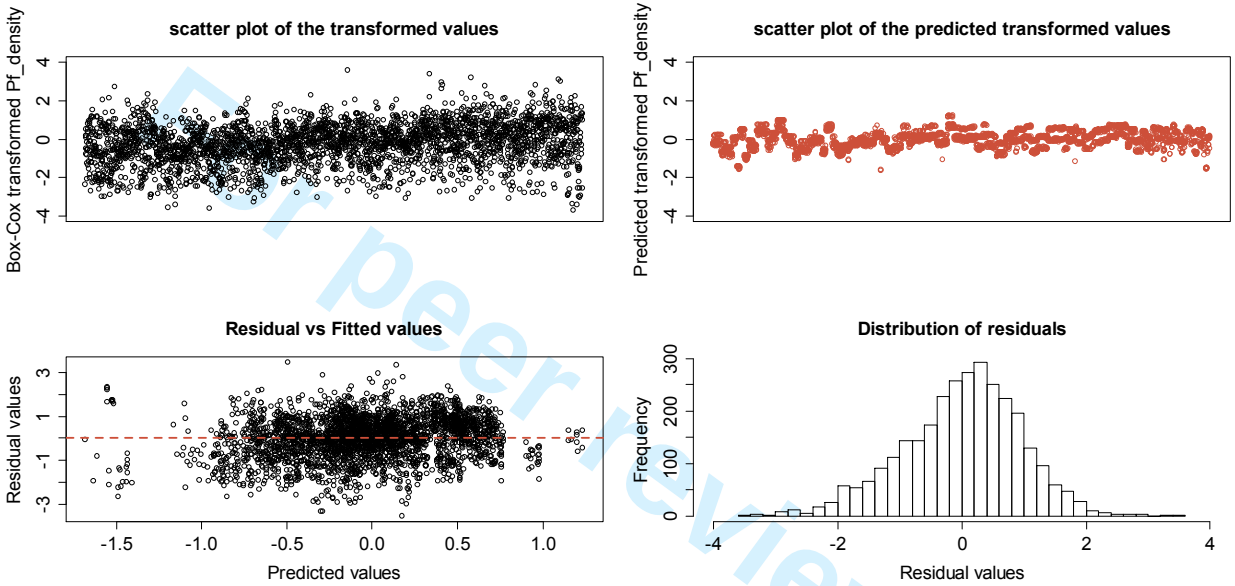
However, we tried the Probit normalization on the log(*pf_density*) to readjust its quantiles to those from a standard normal, and subsequently used the derived standard normal transformation of the log(*pf_density*) as outcome (see Figure S7 below, the three graphs presented in the first row of the graphs panel concern the log(*pf_density*) before Probit normalization and the three in the second row are for after Probit normalization. We can see on the histogram in blue color a good normal distribution of the y variable.

Figure S7. Probit normalization of the log(*pf_density*)



The results we obtained after this Probit normalization of the log(*pf_density*) confirmed the same findings. Also, the corresponding graphical control of the model adequation presented on Figure S8 below, shows a good normal distribution of residuals from this model.

Figure S8. Graphical control of the model adequation after Probit normalization of the log(*pf_density*)



Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium falciparum* malaria

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Article summary

Article focus

- Genetic studies suggest a link between susceptibility to allergy and malaria in Africa
- We hypothesize that atopy increases susceptibility to malaria

Key messages

- Results demonstrate an association between asthma, atopic dermatitis and susceptibility to clinical *P. falciparum* episodes.
- Genetic pre-disposition to asthma or atopic dermatitis impairs the acquisition of clinical immunity to malaria.
- Administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Strengths and limitations

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association.

Abstract

Objectives: To assess the impact of atopy and allergy on the risk of clinical malaria.

Design: A clinical and immunological allergy cross-sectional survey in a birth cohort of 175 children from 1 month to 14 years of age followed for up to 15 years in a longitudinal open cohort study of malaria in Senegal. Malaria incidence data were available for 143 of these children (aged 4 months to 14 years of age) for up to 15 years. Mixed model regression analysis was used to determine the impact of allergy status on malaria incidence, adjusting for age, gender, sickle cell trait and force of infection.

Main outcome measures: Asthma, allergic rhinoconjunctivitis and atopic dermatitis status, the number of clinical *Plasmodium falciparum* malaria episodes since birth and associated parasite density.

Results: Twelve percent of the children were classified as asthmatic and ten percent as having atopic dermatitis. These groups had respectively a two-fold (OR 2.12 95% confidence intervals 1.46 to 3.08; P= 8 $x10^{-5}$) and three-fold (OR 3.15, 1.56 to 6.33; P= 1.3 $x10^{-3}$) increase in the risk of clinical *P. falciparum* malaria once older than the age of peak incidence of clinical malaria (3 to 4 years of age). They also presented with higher *P. falciparum* parasite densities (Asthma: mean 105.3 parasites/µL ±SE 41.0 *vs*. 51.3 ±9.7; P= 6.2 $x10^{-3}$; Atopic dermatitis: 135.4 ±70.7 *vs*. 52.3 ±11.0; P=0.014). There was no effect of allergy on the number of non-malaria clinical presentations. Individuals with allergic rhinoconjunctivitis did not have an increased risk of clinical malaria nor any difference in parasite densities.

Conclusion: These results demonstrate that asthma and atopic dermatitis delay the development of clinical immunity to *P. falciparum*. Despite the encouraging decrease in malaria incidence rates in Africa, a significant concern is the extent to which the increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines, administration to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Introduction

The World Allergy Organization estimates that 40% of the world's population is concerned by allergic diseases.[1] In developing countries where *Plasmodium falciparum* malaria is endemic, prevalence of allergy is significantly lower, but is on the increase.[2] T helper type 2 (Th2) cells, their related cytokines, IgE, eosinophils and mast cells play a major role in allergic inflammation. Orientation of the immune response towards a Th1 profile is crucial for immunity to intracellular pathogens,[3] whereas orientation towards a Th2 profile drives immunity to extracellular pathogens and antigens resulting in class switching giving rise to IgE-producing B cells.[4] An important role of the Th1/Th2 balance in the development of clinical malaria following infection by *P. falciparum* has been suggested by numerous studies.[5-7] Whilst it is recognised that acquired anti-parasite immunity is IgG dependent,[8] It has been suggested that the Th2 bias induced by *P. falciparum* may exacerbate allergy parasite-specific IgE also impact upon the clinical outcome of infection.[8] For example, higher IgE but not IgG levels have been observed in patients with cerebral malaria than those with uncomplicated P. falciparum infection.[9] The role of IgE, however, remains unclear. [10] Likewise, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*.

THowever, the interplay between infectious agents and allergy is unclearambiguous. On the one hand, for example, severe respiratory syncytial virus infection in infants increased the risk of allergic rhinoconjunctivitis and allergic asthma.[11,12] On the other hand, measles,[13] hepatitis A[14] and tuberculosis[15] seemingly reduce atopy. Although, an atopic condition can increase incidence of disease, such as the case for the skin commensal *Staphylococcus aureus* in patients with atopic dermatitis,[16] an atopic tendency *per se* does not generally lead to increased illness from infectious agents.

Genome wide studies have identified chromosomal regions linked to clinical malaria, all of which overlap with those previously identified to be involved in atopic dermatitis, asthma, atopy and IgE levels,[17-19] suggesting that common mechanisms may be involved in both pathologies.[20] Chromosomal region 5q31 that has been repeatedly shown to be associated with control of parasite density and contains a cluster of cytokines, among which IL12B has been previously associated with psoriasis.[21] The other regions, 13q13-q22, 5p15-p13 and 12q21-q23, contain genes involved in innate immunity, notably the interleukin 7 receptor,

and several involved in tumour necrosis factor synthesis [C1q and tumour necrosis factor related protein 3 (C1QTNF3)] and a gene involved in the complement system (C9). [20,18]

Several additional lines of evidence support the concept that susceptibility to malaria and atopy may be related to similar immunological defects. In Ethiopia, a history of malaria was associated with atopy.[22,0] A mouse model for human atopic disease was found to be very susceptible to murine malaria and a major locus for atopic disease mapped close to the region controlling parasite density.[23,1] This region contains several candidate genes that have effects on T-cell function.[23,1]

Moreover, a direct effect of histamine in the malaria pathogenesis has been found using genetic and pharmacological approaches[24,2] and increased levels of histamine are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[25,3,26,4]

To test the hypothesis that allergy impacts upon clinical *P. falciparum* malaria, we performed a clinical allergy cross-sectional study in the family-based longitudinal cohort from Senegal previously used for the genome linkage study[20,18] and analysed the impact of asthma, atopic dermatitis, allergic rhinoconjunctivitis on the incidence of clinical *P. falciparum* episodes and the maximum parasite density during each episode.

Methods

Population and outcome data

The malaria research program conducted in Dielmo village in Senegal has been ongoing since 1990 as described elsewhere.[27,5] In brief, between 1990 and 2008, a longitudinal study involving the inhabitants of the village of Dielmo, Senegal, was carried out to identify all episodes of fever. The study design included daily medical surveillance with systematic blood testing of individuals with fever and examination of 200 oil-immersion fields on a thick blood film for malaria parasites (about 0.5 µL of blood). Each individual was given a unique identification code and details of family ties, occupation, and precise place of residence were recorded on detailed maps of each household with the location of each bedroom. All households were visited daily, absenteeism recorded, and the presence of fever or other

symptoms assessed. We systematically recorded body temperature at home three times a week (every second day) in children younger than 5 years, and in older children and adults in cases of suspected fever or fever-related symptoms. In cases of fever or other symptoms, blood testing was done at the dispensary by finger prick, and we provided detailed medical examination and specific treatment. Parasitologically confirmed clinical malaria episodes were treated according to national guidelines. From 1990 to 2008, four different drug regimens were implemented: Quinine from 1990 to 1994, Chloroquine from 1995 to 2003, Fansidar (sulfadoxine-pyrimethamine) from 2004 to mid-2006 and Artemisinin-based combination therapy (ACT; Amodiaquine- sulfadoxine-pyrimethamine) from mid-2006 to 2008.

Parasite positivity was established as follows. Thick blood films were prepared and stained by 3% Giemsa stain. Blood films were examined under an oil immersion objective at x1000 magnification by the trained laboratory technicians and 200 thick film fields were examined to count the number of asexual and gametocyte parasite stages. Asexual parasite densities (per µL) were calculated by establishing the ratio of parasites to white blood cells and then multiplying the parasite count by 8,000, the average white blood cell count per µL of blood.

Malaria transmission in Dielmo is intense and perennial. We conducted a cross-sectional survey to estimate the prevalence of symptoms related to allergic diseases among 175 children aged from 1 month to 14 years old who were born during the malaria research program.

Both the longitudinal and cross-sectional surveys were approved by the Ministry of Health of Senegal. Informed consent of the volunteers is renewed every year. More specifically for the cross-sectional survey, after informing about the procedures and the purpose of the study, written informed consent was obtained from parents or guardians of children either by signature or by thumbprint on a voluntary consent form written in both French and Wolof, the main local language. Consent was obtained in the presence of the school director, an independent witness.

The family structure (pedigree) was available after a demographic census performed for every volunteer at his adhesion in the project. A verbal interview of mothers or key representatives of the household was used to obtain information on genetic relationships between studied individuals, their children, their parents, and to identify genetic links

among the population. The total pedigree comprised 828 individuals, including absent or dead relatives, composed of ten independent families that can be sub-divided into 206 nuclear families (father – mother couples with at least one child) with an average of 3.6 children each. Genetically related nuclear families occur because of multiple marriages and marriages among related individuals. Previous typing with microsatellites has enabled the construction of a pedigree based on Identity-by-Descent using MERLIN.[2018,286] The mean coefficient of inbreeding is 0.0008. Newborns since this original genetic analysis were added to the family of the parents in question. The 143 children, with both allergy and malaria data, belonged to 61 nuclear families and comprised 30 singletons, 102 siblings and 11 half-sibs (yielding 55 half-sib pairs). The mean genetic relatedness (by pedigree) of the 143 children is 0.0114 (range: 0.0013 to 0.022).

P. falciparum *clinical episodes*

P. falciparum malaria clinical episode phenotypes analysed were: (i) clinical *P. falciparum* infections treated with anti-malarial therapy and (ii) the highest parasite density during the *P. falciparum* clinical episode. A clinical *P. falciparum* episode was defined as a clinical presentation with fever (axillary temperature ≥ 37.5°C) and/or other clinical signs suggestive of malaria associated with a thick blood smear positive for *P. falciparum* and that was treated with anti-malarial therapy. Repeated clinical malaria presentations within 15 consecutive days were not considered to be independent and were excluded from the analyses, unless there was a negative thick blood smear between two clinical presentations. We also excluded observations in any trimester for which the individual was not present for at least one third of the time.

We calculated the quarterly incidence rate of clinical *P. falciparum* episodes in children below the age of 15 years as the ratio of the total number of clinical *P. falciparum* episodes during the trimester divided by the total number of person-trimesters surveyed. Incidence rate is expressed as cases per 100 person-trimesters (see Supplementary Figure S1). This rate was used in the analysis to approximate the force of infection (exposure level) within the targeted population at the time of a given clinical *P. falciparum* episode.

The total number of clinical presentations per trimester that were not attributable to *P. falciparum* was tabulated. Repeated non-malaria presentations within seven consecutive days were not considered to be independent and were excluded.

Allergic diseases and atopic status

The International Study of Asthma and Allergies in Childhood (ISAAC) diagnostic criteria have been shown to be reproducible, adequate and able to discriminate children with allergic diseases in different areas of the world.[2] The standardized ISAAC questionnaire originally written in English was translated into French in compliance with ISAAC guidelines[2~~97~~], adapting it to the usual local customs following advice from local clinicians and paediatric allergologists (Acknowledgements and Technical Appendix). The adequacy and reliability of the translated questionnaire had been previously confirmed by a pilot study on 30 randomly selected children in the same community. The questionnaire was completed by specially trained health workers during an oral interview conducted in Wolof with children and their mothers or guardians.

To assess the prevalence of allergic diseases in children, we used the positive and negative predictive values of the ISAAC questionnaire diagnosis criteria developed for subtropical countries.[30~~28~~] Each question was scored according to the medical diagnosis of paediatricians and paediatric allergologists. Positive or negative answers were thus graded on the basis of symptom sensitivity, specificity, frequency, location or early onset. For each allergic disease, three categories of symptom severity, *severe*, *moderate*, and *none*, were defined as follows:

Asthma – severe symptoms if the child had "wheezing or whistling in the chest before the age of two years" and "more than three times" *or* severe enough to "limit his/her speech"; *moderate* symptoms if the child had "wheezing or whistling in the chest before the age of two years" and "in the past 12 months"; and *none* otherwise.

Allergic rhinoconjunctivitis – severe symptoms if the child had "sneezing, runny or stuffy nose in the past 12 months" and "more than five times a year", and "itchy, watery eyes or tropical endemic limboconjunctivitis (TELC) in the past 12 months"; *moderate* symptoms if the child had "sneezing, runny or stuffy nose in the past 12 months", and "itchy, watery eyes or TELC in the past 12 months"; and *none* otherwise.

Atopic dermatitis – severe symptoms if the child had "scaly or exudating, crusted and pruritic patches in the past 12 months" and "affecting any of the following characteristic areas: face, around the ears or eyes, folds of armpits or elbows or groin, behind the knees, under the buttocks", and "onset of symptoms before the age of two years"; *moderate* symptoms if the

child had “scaly or exudating, crusted and pruritic patches in the past 12 months” and “affecting any of characteristic areas (see above)”, and “onset of symptoms before the age of four years”; and *none* otherwise.

The inter-relationships between variables reflecting the severity of symptoms of the three allergic diseases were used to identify children at high risk of atopy. The *high probability* group was defined by the prevalence of at least one of any *severe* symptoms or two of any *moderate* symptoms. The *probable* group was defined as those with *moderate* symptoms from one of the three allergic diseases and remaining children were classified in the *unlikely* group.

Helminths

Helminthic infections are common in this region and are known to modify the clinical course and outcome of both allergic diseases and malaria.[31,32] We therefore carried out a helminth survey for 91 individuals present during the cross-sectional survey. Diagnosis was performed by stool examination by microscope and by the Kato technique to search for the presence of *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*), whipworm (*Trichuris trichiuria*), *Schistosoma mansoni*, and *Strongyloides stercoralis*. Examination for pinworms (*Enterobius vermicularis*) was performed by the anal scotch-test. An anti-helminthic treatment was proposed for all infested individuals.

Immunoglobulin E titres

Specific IgE titres were measured by ELISA as previously described.[33] A panel of allergens of potential pertinence to the three classes of allergy was used: (i) Salivary gland extracts (SGE) of two mosquito species present in the study cohorts, *Aedes aegypti* and *Anopheles gambiae sensu stricto*, and (ii) *P. falciparum* parasite extract were prepared as previously described[31]; (iii) House dust mite spp. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*; (iv) a mix of pollen allergens from five ubiquitous graminae spp. [Cock’s-foot (*Dactylis glomerata*), Timothy grass (*Phleum pratense*), Sweet Vernal grass (*Anthoxanthum odoratum*), Perennial ryegrass (*Lolium perenne*), Kentucky Bluegrass (*Poa pratensis*)] (all from Stallergenes, France).

Statistical analysis

Statistical analyses were performed using R version 2.12.0 (The R Foundation for Statistical Computing, Vienna, Austria). To address the effect of allergic status on the risk of clinical *P. falciparum* episodes, we performed Generalized Linear Mixed Models (GLMM) extended to pedigree data using the *pedigreemm* package for R to account for the non-independence of individuals because of family relationships, shared house and for repeated measures from the same individual (Technical Appendix). Correlated individual effects due to familial relationships were taken into account by using the pedigree-based genetic relatedness matrix that contains the genetic covariance among all pairs of individuals in the study cohort and is calculated using the pedigree information.[34~~2~~] Shared house and repeated measures from the same individual were modelled as random effects. All random effects were assumed to be normally distributed, and conditional on these random effects, the dependent variable had: (i) a Binomial distribution when the studied phenotype was the occurrence of a clinical *P. falciparum* episode treated with anti-malarial therapy during a trimester, (ii) a Gaussian distribution when the studied phenotype was the logarithm of the maximum parasite density during a given clinical *P. falciparum* episode, and (iii) a Poisson distribution when the studied phenotype was the number of non-malaria episodes per trimester. The effects of allergy disease classes on these dependent variables were modelled as fixed effects. Allergy classes were reduced to two levels, *Severe* or *moderate vs*. *none* for analyses of asthma, atopic dermatitis and allergic rhinoconjunctivitis and *high probability vs*. *probable* and *unlikely* for atopic tendency. Co-variables included sickle cell trait[33~~1~~], gender, number of days present on site during the trimester, trimestrial incidence of *P. falciparum* and age. Age was initially analysed as a continuous covariate. To assess the age-specific effect of allergy, age was categorised into two levels (<3.5 years of age and ≥3.5 years of age, based on the age of peak clinical incidence) and allergy class was nested within age class. The age threshold was varied from 1.5 years to 5.5 years of age and the data re-analysed to assess at which age there was the strongest effect. The association of allergy classes with IgE levels was analysed by box-cox transforming the data and fitting a GLMM with a normal distribution.

Results

Of the 205 eligible children aged under 15 years involved in the family-based longitudinal study, 175 (85.4 %) participated in the cross-sectional survey to assess the prevalence of related symptoms of allergic diseases. All eligible children present at the time of the survey were included; no explicit refusal to participate was recorded. The study cohort was aged from 1 month to 14 years 11 months. The sex-ratio (male/female) was 0.94.

From 1994 until 2008, 143 of the children participating in the cross-sectional survey were present for at least 31 days in any trimester during the study period generating a total of 3,093 person-trimesters of presence (Supplementary Table S1). There were 2,065 treated *P. falciparum* clinical episodes (per individual: median 11, range 0-47)(Supplementary Table S2). The age peak of incidence of *P. falciparum* episodes occurred at 3 to 4 years of age (Figure 1). There were 1,868 non-malaria episodes (median 12, range 0-37) (Table S2). These non-malaria clinical presentations were associated with headache (38 %), chills (32 %), cough (13 %), vomiting (11 %) and diarrhoea (6 %).

The prevalence of moderate or severe asthma symptoms was respectively 2.3 % and 10.3 % (Table 1). The prevalence of moderate or severe allergic rhinoconjunctivitis symptoms was respectively 6.3 % and 10.3 %. The prevalence of moderate or severe atopic dermatitis symptoms was respectively 6.3 % and 2.9 %. On the basis of symptom severity, an atopic tendency was estimated to be unlikely for 68.0 %, probable for 9.1 % and highly probable for 22.9 % of the 175 children. The frequency of each allergy class in children for whom malaria data were available is shown in Table S1.

The risk of treated clinical *P. falciparum* infections was higher for children with high probability of atopy (OR 1.65, 95% confidence intervals 1.20 to 2.26; P=0.002) (Table 2), after adjusting for age, sickle cell trait and the exposure level. Gender was not found to be significant. Analysing the impact of atopy in children younger and older than the peak age of clinical incidence (3 to 4 years old), revealed that atopy increased the risk of *P. falciparum* episodes in children at an age greater than 3.5 years (OR 2.02, 1.39 to 2.93; $P=2x10^{-4}$), but not in children of age prior to the peak clinical incidence (OR 1.38, 0.92 to 2.08; P=0.124) (Table 2). This increased risk resulted in an ever increasing cumulative number of *P. falciparum* episodes with age beyond that of peak clinical incidence (Figure 2. See supplementary Figure S2 for model predictions for comparison).

Analysis by allergy category revealed that asthma (severe or moderate) increases the risk of *P. falciparum* episodes (OR 2.12, 1.46 to 3.08; P= 8 x10^{-5}) and this again only in children of age greater than 3.5 years old (OR 2.33, 1.50 to 3.61; P= 1.5 x10^{-4}). Atopic dermatitis increased the risk of clinical malaria in children older (OR 3.15, 1.56 to 6.33; P= 1.3 x10^{-3}) but not younger than 3.5 years of age (Table 2). Allergic rhinoconjunctivitis was not associated with increased risk of clinical malaria at any age (Table 2). The impact of atopy, asthma and atopic dermatitis can be clearly seen in the ever-increasing number of cumulative *P. falciparum* episodes beyond the age of the onset of clinical immunity in the population, 3.5 years of age (Figure 2). There is no difference in the number of clinical malaria episodes prior to this age in individuals with or without an allergic condition. Analysis using different age thresholds (from 1.5 to 5.5 years of age) revealed similar OR for thresholds of 2.5, 3.5 and 4.5 years of age. The maximum OR for increased malaria occurred in children older than 4.5 years of age and with atopy or atopic dermatitis, whereas for the asthma group it occurred in children after 3.5 years of age (Supplementary Table S3).

There was no impact of any allergic disease on the number of non-malaria episodes by trimester (Supplementary Table S4).

The impact of atopy, asthma and atopic dermatitis on the maximum *P. falciparum* parasite density during a given clinical malaria episode mirrored that of the risk of *P. falciparum* episodes. Parasite density was significantly higher for children with allergic disease older than 3.5 years of age (Table 3 and supplementary Figure S3 for residuals of the fitted model). As the log-transformed data were left skewed, we additionally analysed using box-cox transformation and probit normalization of the data. The results were qualitatively the same (Supplementary text and Figures S4-S8). Allergic rhinoconjunctivitis had no impact on the parasite density (Table 3). Analysis using different age thresholds yielded ~~the same pattern~~similar qualitative conclusions as seen with the number of clinical episodes (Table S3).

Individuals with moderate or severe symptoms of atopic dermatitis had significantly higher specific IgE titres against *Ae. aegypti* (P=0.004) and *An. gambiae* SGE (P<0.001). There were no detectable specific anti-*P. falciparum* IgE. Individuals with moderate or severe symptoms of allergic rhinoconjunctivitis did not have significantly higher IgE titres against the tested graminae (P=0.28), although titres decreased with age (P=0.035). There was also no effect of

asthma on IgE titres against the house dust mite spp. tested (*D. farinae* P=0.60 & *D. pteronyssinus* P=0.27).

Only five individuals were infested with helminths (two *Ancylostoma*, one *Strongyloides*, one *Trichuris* and one *Enterobius*).

Discussion

Principal findings

Establishing the allergic status of children up to the age of 15 years old followed for malaria since birth, revealed an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

Strengths and weaknesses of the study

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association. In addition, although allergy diagnosis for children under 2 years of age is not considered reliable, there were only 15 individuals under 2 at the time of the allergy study of the 143 for whom malaria and allergy data were available.

Meaning of the study

Under intense malaria transmission, after repeated exposure to the parasite, children develop a clinical immunity[3,5], whereby they tolerate elevated parasite densities without showing clinical symptoms. In this cohort, the population mean onset of clinical immunity occurred at 3 to 4 years of age. Although clinical immunity is accompanied by a reduction in parasite density, effective anti-parasite immunity develops much more slowly[3,6,4] with individuals achieving a state of premunition, whereby they maintain low-grade parasite densities in an asymptomatic state.[3,7,5] We show here that children with clinically defined

asthma or atopic dermatitis ~~had a two to three-fold~~have an increased ~~in the~~ risk of presenting with *P. falciparum* malaria episodes requiring treatment once passing the age of peak clinical incidence. They also had higher parasite density during clinical episodes, suggesting a reduced ability to control parasite replication. The observed increase in clinical incidence of malaria in patients with asthma or atopic dermatitis is not likely to be the result of increased frailty of such individuals; these individuals did not come more frequently to the clinic with non-malaria symptoms. Our previous genome linkage study identifying chromosomal regions[20~~18~~] associated with malaria that overlap with those previously shown to be linked to asthma/atopy suggests that there may be a shared genetic basis to these pathologies rather than any causative effect of one on the other. This is consistent with the increased susceptibility to malaria of mouse atopic models.[2~~31~~]

Comparison with other studies

A previous study in Ethiopia (East Africa) found that a history of malaria (yes/no) increased risk of atopic dermatitis in 306 cases compared to 426 controls as characterized using the ISAAC questionnaire.[2~~20~~] The only other epidemiological study that has previously examined the link between malaria and atopy[3~~86~~] also interpreted the result from the perspective of the impact of malaria on atopy. They examined the re-infection rate with *P. falciparum* over a 5-year period in 91 children that were subsequently classified as atopic or not using skin prick tests (SPT) with house dust mite antigen. Their conclusion was that, as with measles[13~~1~~] and tuberculosis[15~~3~~], malaria infection reduces atopy. However, the study lacked previous infection data since birth of the participating individuals and focussed on atopy as determined by SPT against a single allergen. The case-control study of atopic dermatitis risk factors cited above found no overall association between allergen skin sensitization and atopic dermatitis. We also found no evidence of increased IgE titres against house dust mites in the asthmatic or atopic dermatitis groups or against grass pollen in individuals with allergic rhinoconjunctivitis. Such differences likely reflect the different IgE reactivity profiles due to differences in allergen exposure in Africa.[39~~7~~] There was no evidence of anti-parasite IgE in this cohort of children. We previously showed that circulating anti-parasite IgE titres were strongly positively correlated with anti-mosquito saliva IgE, but became undetectable following malaria exposure, potentially being bound to effector cells.[33~~1~~] Only mosquito

saliva, a known major local allergen, induced a specific IgE response at significantly higher titres in individuals with atopic dermatitis.

Although the immune effectors of clinical immunity are still poorly defined, there is strong evidence that acquired anti-parasite immunity is IgG-dependent[38] and cytophilic immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by opsonisation and/or by Antibody Dependent Cellular Immunity play an important role in premunition.[375] The higher parasite density during symptomatic episodes observed in the asthma group suggests impaired development of acquired immunity. Impaired acquisition of immunity to malaria in children with asthma or atopic dermatitis may stem from their imbalanced Th1/Th2 response. Indeed, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*. Dendritic cells that are oriented to a Th2 phenotype are more susceptible to orient the acquired immune response towards a Th2 profile.[4039] Orientation of the immune response towards a Th2 profile by asthma or atopic dermatitis would result in a poor Th1 response (and hence development of protective IgG immunoglobulins), considered to be the dominant arm of the immune response enabling resistance to infectious disease in children.[410]

Many studies have revealed an important role of histamine, a key downstream effector molecule in allergic reaction, in the outcome of a malaria parasite infection.[242-264,421-454] Moreover, reports indicate that components of the innate immune system, including eosinophils, basophils, and mast cells (MCs), could play important roles in the pathogenesis of malaria.[421] Increased levels of histamine in plasma and tissue, derived from basophils and MCs, notably following stimulation by IgE through the high affinity receptor FcεR1, are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.[253,264] Chlorpheniramine, a HR1agonist reversed resistance to chloroquine and amodiaquine both *in vivo* and *in vitro*.[432] Moreover, astemizole, another HR1 agonist, was identified as an anti-malarial agent in a clinical drug library screen.[443] Finally, *P. falciparum* produces translationally controlled tumor protein, which is a homolog of the mammalian histamine-releasing factor that causes histamine release from human basophils.[454]

Further research

Our results provide the first birth cohort study addressing the link between malaria and allergic diseases. They contribute to a growing body of evidence that the pathologies are related. ISAAC has revealed a steady but significant increase in prevalence rates of asthma and allergic diseases in Africa. Whilst the majority of studies have focused on large cities, there is increasing urbanization throughout Africa, as well as improved access to primary health care in many areas. A key concern for ISAAC is the extent to which such societal evolution will result in an increase in allergic diseases. Increased urbanization in sub-Saharan Africa is changing the epidemiology of malaria and although resulting in a decrease in risk, will result in more severe clinical malaria in older individuals.[4,5,4,6] Moreover, a large consumption of anti-malarial drugs in the urban areas provides substantial drug pressure fostering, the selection of drug-resistant parasites. Despite the encouraging recent decrease in malaria incidence rates, even in rural areas, an additional significant concern is the extent to which such an increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines[47,48], administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials[49,8] and alleviate the non-infectious consequences of atopy. Clinical intervention studies should be envisaged.

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What is already known on this topic

There are several reports of the beneficial effects of anti-histamines for malaria chemoprophylaxis[2,42-2,64,48,7] as well as our previous work[20,18] showing that chromosomal regions associated with malaria are also linked to allergy and atopy.[1,75-1,97] There are two epidemiological studies showing opposite effects of malaria on atopy.[2,20,3,86]

What this study adds

Using a longitudinal malaria study birth cohort, we identified an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred only after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

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Contributors: LB, SM, and RP made substantial contributions to the concept and design of the study. MH, HB, BG, SB, FDS, and AT were involved in acquisition of the data. CL, AF, OMP, AS and RP contributed to the analysis and interpretation of the data. MH, CL, HB, BG, SB, FDS, AF, AT, LB, OMP, SM, AS and RP critically reviewed the report and approved its final version for submission. All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. MH and RP are guarantors.

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: The allergy study was approved by the Senegalese National Ethics committee (2009/N°46). Renewed approval of the longitudinal malaria study was obtained from the same committee (2006/N°969).

Data sharing: The allergy database will be made available on-line. The longitudinal malaria data set will be made available following discussion with the coordinators of the three Institutes that govern the dataset through contact with the corresponding author.

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Table 1 Classification of Asthma, Allergic rhinoconjunctivitis, Atopic dermatitis and overall Atopic status according to ISAAC questionnaire in children aged 0-14 from a malaria birth cohort. N is total number of children examined and n-malaria represents those for whom malaria data were recorded. F is the number of females and M the number of males.

	N (F/M)	%	n-malaria (F/M)
Asthma symptoms			
None	153 (73/80)	87.43	125 (59/66)
Moderate	4 (1/3)	2.29	4 (1/3)
Severe	18 (6/12)	10.29	14 4/10)
Rhinoconjunctivitis symptoms			
None	146 (64/82)	83.43	120 (52/68)
Moderate	11 (8/3)	6.29	9(6/3)
Severe	18 (6/12)	10.29	14 (6/8)
Atopic dermatitis symptoms			
None	159 (75/84)	90.86	128 (60/68)
Moderate	11 (1/10)	6.29	11 (1/10)
Severe	5 (4/1)	2.86	4 (3/1)
Atopic tendency			
Unlikely	119 (56/63)	68.00	97 (46/51)
Probable	16 (8/8)	9.14	14 (6/8)
Highly probable	40 (16/24)	22.86	32 (12/20)

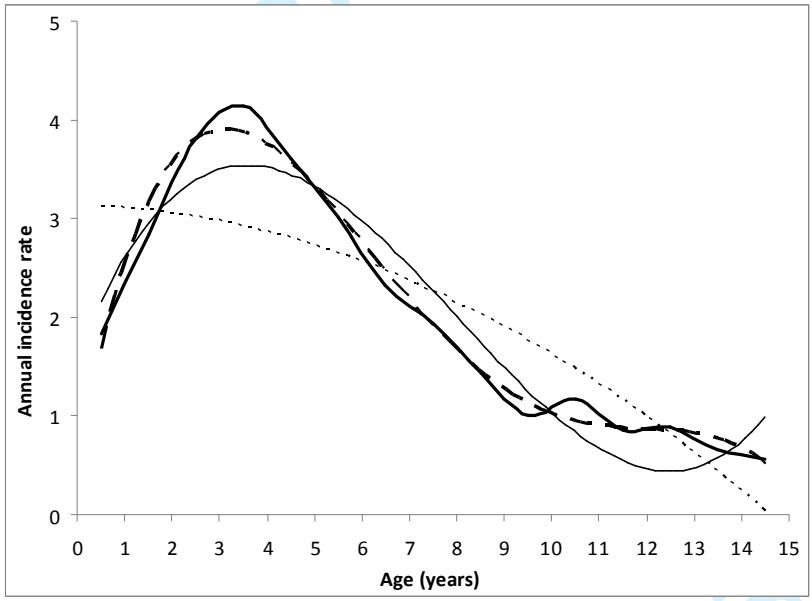
Table 2 Impact of allergy status on risk of *P. falciparum* clinical episodes. Shown are the *P* values and adjusted Odds Ratios with 95% confidence intervals calculated from the mixed model analyses. Values for the covariables Age (≥3.5 years of age compared to <3.5 years of age), Trimestrial incidence of *P. falciparum* clinical episodes and HbAS (beta-globin sickle cell trait; AS compared to AA) are those from the Asthma model analysis. For clarity significant co-variables are shown in bold.

	Age groups <3.5 years>	ORa	95% Confidence Intervals		*P* value
			Lower	Upper	
Atopy	**Both**	**1.65**	**1.20**	**2.26**	**2.0×10^{-3}**
	<3.5	1.38	0.92	2.08	0.124
	≥3.5	**2.02**	**1.39**	**2.93**	**2.1×10^{-4}**
Asthma	**Both**	**2.12**	**1.46**	**3.08**	**8.0×10^{-5}**
	<3.5	1.50	0.90	2.50	0.122
	≥3.5	**2.33**	**1.50**	**3.61**	**1.5×10^{-4}**
Atopic dermatitis	Both	1.05	0.65	1.70	0.842
	<3.5	0.84	0.49	1.46	0.539
	≥3.5	**3.15**	**1.56**	**6.33**	**1.3×10^{-3}**
Rhinoconjunctivitis	Both	0.96	0.65	1.41	0.818
	<3.5	1.05	0.64	1.72	0.853
	≥3.5	0.95	0.60	1.52	0.834
Age ≥3.5		**0.48**	**0.40**	**0.57**	**2.7×10^{-15}**
Trimestrial incidence		**1.01**	**1.00**	**1.01**	**1.8×10^{-6}**
HbAS		**0.24**	**0.12**	**0.47**	**3.7×10^{-5}**

Table 3 Impact of allergy status on the maximum *P. falciparum* parasite density during a clinical malaria episode. Shown are the back-transformed mean parasite densities per microlitre and standard errors (SEM) estimated from the GLMM analyses after taking into account the other co-variables. Significantly different effects are shown in bold for clarity.

Allergic condition	Age groups	Allergic status (No/Yes)	Mean parasite density	SEM	*P* value
Atopy	**Both**	**N**	**76.3**	13.8	
		Y	**131.0**	36.4	**0.0158**
	<3.5	N	114.3	23.7	
		Y	171.1	56.0	0.148
	≥3.5	**N**	**48.4**	9.8	
		Y	**114.8**	37.1	**$9.5x10^{-4}$**
Asthma	**Both**	**N**	**78.1**	14.4	
		Y	**148.5**	44.3	**$3.8\ x10^{-3}$**
	<3.5	N	114.8	24.3	
		Y	171.9	74.5	0.167
	≥3.5	**N**	**51.3**	9.7	
		Y	**105.3**	41.0	**$6.2\ x10^{-3}$**
Atopic dermatitis	Both	N	82.6	15.0	
		Y	93.9	38.9	0.605
	<3.5	N	122.6	25.5	
		Y	133.9	63.5	0.425
	≥3.5	**N**	**52.3**	11.0	
		Y	**135.4**	70.7	**0.014**
Rhinoconjunctivitis	Both	N	81.5	14.8	
		Y	111.4	39.0	0.570
	<3.5	N	118.8	25.1	
		Y	166.3	69.9	0.537
	≥3.5	N	54.6	11.3	
		Y	80.9	33.7	0.327

Figure 1 Annual incidence rate of clinical *P. falciparum* episodes per 100 children (bold line). In order to overcome the fluctuations of the annual incidence rate, we fit second (dotted line), third (dashed line) and fourth (solid line) degree polynomial trend lines to the data (bold line). The corresponding R-squared values are 0.70, 0.91 and 0·99 respectively indicating an accurate fit for third and fourth order polynomials. The inflexion on these two trend lines indicates the onset of acquisition of clinical immunity at approximately 3 to 4 years of age.



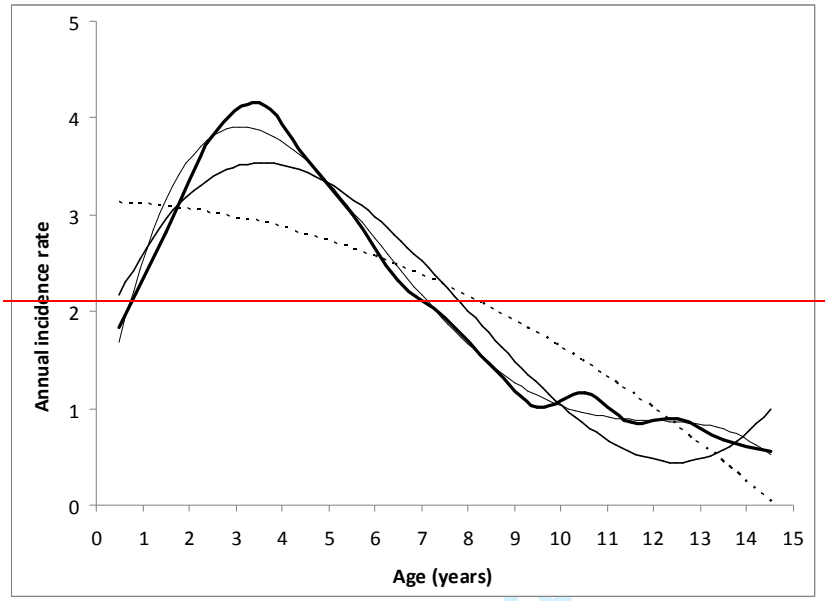
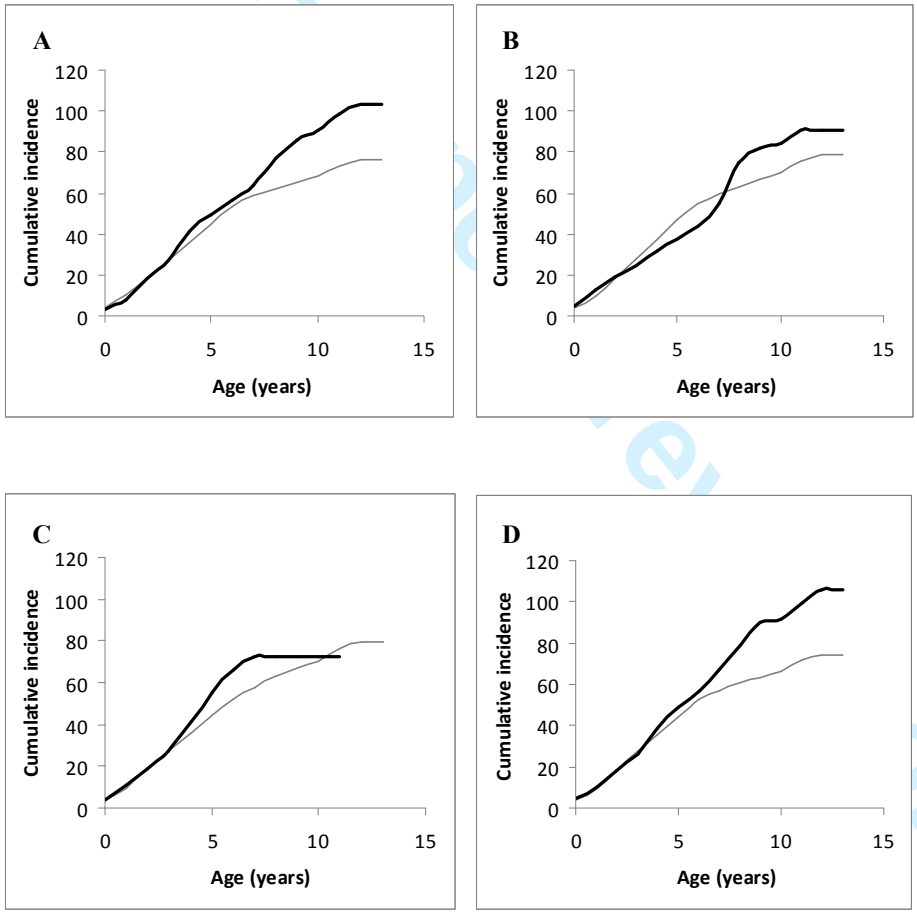


Figure 2 Mean cumulative number of *P. falciparum* clinical episodes with age for the (A) Asthma, (B) Rhinoconjunctivitis and (C) Atopic dermatitis classes and overall Atopy class (D) (bold lines) compared to individuals without symptoms of each respective allergy type (thin lines). In all cases moderate and severe classes are combined and compared to individuals without allergy symptoms. Note there are no children older than 11 years of age with Atopic dermatitis.



INSTITUT PASTEUR	Unit of Functional Genetics of Infectious Disease	Coding :	
	ENREGISTREMENT	03/MM/YYYY	Version : 1
	ALLERGY MODIFIED ISAAC QUESTIONNAIRE		

Place of study	***Research Institute responsible***
Technician X	*Name of Institute*
Technician X	*Principle investigator*
Technician X	*Project manager*
☎ +	☎ +

IDENTIFICATION

Validation zone

Date of questionnaire : |__|__| |__|__| |__|__|__|__| dd/*mm/yyyy* — |__|__| |__|__| |__|__|__|__| DTEQUE

Name of investigator :……

Name of study supervisor :……

Child :

First and last name of child :

Identification code of child : — |__|__| |__|__| IDENF

Date of birth : — |__|__| |__|__| |__|__|__|__| DOB

Sex : — |__| SEXE

Village/town : — |__| VILLAGE

Identification code of Questionnaire : — |__| |__|__| |__| |__|__||__|__| IDQUES

Identification code of father : — |__|__| |__|__| IDFA

Identification code of mother : — |__|__| |__|__| IDMO

Weight : |__|__|,|__| **(kg)** — |__|__|,|__| (Kg) WEIGHT

Height : |__|__|__|,|__| **(cm)** — |__|__|__|,|__| (cm) HEIGHT

Mid Upper Arm Circumference: |__|__| **(cm)** — |__|__| (cm) MUAC

Person questioned :

Last name of person questioned :…… — …… NAMEPQ

First name of person questioned :…… — …… LASTNAMEPQ

Relationship to child : Mother ☐1 Father ☐2 Brother/Sister ☐3 Grand-parents ☐4 Other ☐5 — |__| RELCHILD

If other, define : …… — …… OTHERREL

FACTORS PREDISPOSING ATOPY

First days of life : Consultation of health records of child and maternity records of mother

1. How much did your child weigh at birth ?

<1500 g ☐1 — [2500-3500[g ☐4

[1500-2000[g ☐2 — ≥ 3500 g ☐5

[2000-2500[g ☐3 — Record not found/NSP ☐9

|__| BIRTHWEIGH

2. Until what age did your child breastfeed (**exclusively or mixed**) ?
Corresponds to the age of weaning of child

< 6 months ☐1 6 – 12 mths ☐2 12 – 24 mths ☐3 >24 mths ☐4 NSP ☐9

|__| AGEWEAN

3. Until what age did your child breastfeed **exclusively** without ever taking other aliments (fruits, vegetables, rice, meat, fish, etc.) or liquids (powdered milk, cow or goats milk, fruit juice, water, etc.) ? < 6 months ☐$_1$ 6 – 12 mths ☐$_2$ 12 – 24 mths ☐$_3$ NSP ☐$_9$		|__|	AGEBREAST
Illness and vaccination : Consultation of health records of child			
1. Has your child enfant had the following illnesses?			
Malaria :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	MALAR
Tuberculosis treated :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	TUBTRT
Helminths (oxyures, ascaris, taenia, etc.) :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	HEMINTH
Amoeba :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	AMOEBA
Measles :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	MEASLES
2. Against what illnesses is you child vaccinated?			
Yellow fever :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACFJ
Hepatitis B :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACHEPB
Measles :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACMEASLE
Mumps :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACMUMPS
Rubella :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACRUBEL
Tuberculosis/BCG :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACTUB
Diphtheria/Tetanus/Pertussis/Poliomyelitis :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACDTCP
Typhoid :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACTY
Meningitis :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACMENIN
Haemophilus influenzae type B (HiB) :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	VACHIB
Habitation :			
1. Which of these animals / insects can be found in the **rooms** where your child lives (today and/or during his first year of life) ?			
Dogs in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	DOGTODAY
Dogs in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	DOG01YR
Cats in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	CATTODAY
Cats in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	CAT01YR
Sheep in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	SHEEPTODAY
Sheep in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	SHEEP01YR
Goats in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	GOATODAY
Goats in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	GOA01YR
Chicken, ducks in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	CHICTODAY
Chicken, ducks in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	CHIC01YR
Rodents (rats, mice, etc.) in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	RODTODAY
Rodents (rats, mice, etc.) in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	ROD01YR
Cockroaches in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	COCTODAY
Cockroaches in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	COC01YR
Other in rooms today :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	OTHTODAY
Other in rooms 0-1yr :	☐$_0$ No ☐$_1$ Yes ☐$_9$ NSP	|__|	OTH01YR
If Others, define :……		……	NAMEOTH
2. Which of these animals could be in **contact** with your child **at least once per week**			

(today and/or during his first year of life) ?

Contact with Dogs today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CDOGTODAY
Contact with Dogs 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CDOG01YR
Contact with Cats today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCATODAY
Contact with Cats 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCAT01YR
Contact with Sheep today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CSHEEPTODAY
Contact with Sheep 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CSHEEP01YR
Contact with Goats today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CGOATODAY
Contact with Goats 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CGOA01YR
Contact with Chicken, Ducks today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCHICTODAY
Contact with Chicken, Ducks 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCHIC01YR
Contact with donkeys, horses today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CHORSTODAY
Contact with donkeys, horses 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CHORS01YR
Contact with Cows, zébus today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCOWTODAY
Contact with Cows, zébus 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CCOW01YR
Contact with Rodents (rats, mice, etc.) today :	☐0 No ☐1 Yes ☐9 NSP	|__|	CRODTODAY
Contact with Rodents (rats, mice, etc.) 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	CROD01YR
Contact with Other today :	☐0 No ☐1 Yes ☐9 NSP	|__|	COTHTODAY
Contact with Other 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	COTH01YR
If Others, define :……		……	NAMEOTHC

3. Which of these aliments are usually stocked in the rooms where your child lives ?

Millet kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	MIL
Sorghum kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	SORG
Maize kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	MAIZ
Rice kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	RICE
Wheat kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	WHEA
Biscuits, pasta kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	BISCUI
Manioc (root, flour) kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	MANIOC
Cashew nut, ground nut kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	NUTP
Curdled milk kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	MILKCURD
Dried leaves (mint, quinquiliba, baobab, etc.) :	☐0 No ☐1 Yes ☐9 NSP	|__|	LEAF
Other aliments kept in room :	☐0 No ☐1 Yes ☐9 NSP	|__|	OTHALIM
If Others, define :……		……	NAMEOTHAL

What is the type of roofing of the rooms where your child lives (today and during the first year of life) ?

Corrugated metal roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	RMETTODAY
Corrugated metal roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RMET01YR
Thatched roof today:	☐0 No ☐1 Yes ☐9 NSP	|__|	RTHATDAY
Thatched roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RTHAT01YR
Wooden roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	RWOOTODAY
Wooden roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RWOO01YR
Cement roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	RCEMTODAY
Cement roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RCEM01YR
Plaster roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	RPLATODAY
Plaster roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	RPLA01YR
Other type of roof today :	☐0 No ☐1 Yes ☐9 NSP	|__|	ROTHTODAY

Other type of roof 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| ROTH01YR
If other, define :.....		..... NAMEOTHR

4. Which of these objects are in the room where your child sleeps (today and during the first year of life) ?

Mattress in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| MATRTODAY
Mattress in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| MATR01YR
Bednet in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| BEDNTODAY
Bednet in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| BEDN01YR
Wardrobe in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| WARDTODAY
Wardrobe in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| WARD01YR
Chest, trunk in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| CHESTODAY
Chest, trunk in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| CHES01YR
Table in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| TABPTODAY
Table in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| TABP01YR
Chair in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| CHPTODAY
Chair in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| CHA01YR
Carpet, rug in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| CARPTODAY
Carpet, rug in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| CARP01YR
Matting in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| MATPTODAY
Matting in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| MATP01YR
Curtains in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| CURTTODAY
Curtains in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| CURT01YR
Malagasy fire in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| FIRTODAY
Malagasy fire in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| FIR01YR
Other objects in room today :	☐0 No ☐1 Yes ☐9 NSP	|__| OTHOBTODAY
Other objects in room 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| OTHOB01YR
If other, define :.....		..... NAMEOTHOB

5. On what type of bedding does your child sleep (today and during the first year of life) ?

Foam mattress today :	☐0 No ☐1 Yes ☐9 NSP	|__| FMATRTODAY
Foam mattress 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| FMATR01YR
Plant fibre mattress (straw, etc.) today :	☐0 No ☐1 Yes ☐9 NSP	|__| PLFMATRTODAY
Plant fibre mattress (straw, etc.) 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| PLFMATR01YR
Wool mattress today :	☐0 No ☐1 Yes ☐9 NSP	|__| WOMATRTODAY
Wool mattress 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| WOMATR01YR
Feather mattress today :	☐0 No ☐1 Yes ☐9 NSP	|__| FEATHMTODAY
Feather mattress 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| FEATHM01YR
Plastic matting today :	☐0 No ☐1 Yes ☐9 NSP	|__| PLMATTODAY
Plastic matting 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| PLMAT01YR
Plant fibre matting (straw, etc.) today :	☐0 No ☐1 Yes ☐9 NSP	|__| PLFMATTODAY
Plant fibre matting (straw, etc.) 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| PLFMAT01YR
Other type of bedding today :	☐0 No ☐1 Yes ☐9 NSP	|__| OTHBEDTODAY
Other type of bedding 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__| OTHBED01YR
If other, define :.....		..... NOMAUTLI

6. Does your child sleep on a pillow ? ☐0 No ☐1 Yes ☐9 NSP \|__\| PILLOW

If **No**, go to question **8**

If **Yes**, what type of pillow is it ?

Foam :	☐0 No ☐1 Yes ☐9 NSP	|__|	PILLF
Synthetic fibres:	☐0 No ☐1 Yes ☐9 NSP	|__|	PILLSYN
Plant fibres (straw, etc.) :	☐0 No ☐1 Yes ☐9 NSP	|__|	PILLPLF
Feather :	☐0 No ☐1 Yes ☐9 NSP	|__|	PILLFEATH
Other type of pillow :	☐0 No ☐1 Yes ☐9 NSP	|__|	OTHPILL
If other, define :.....		.....	NAMEOTHPILL

7. Do people smoke in the room where your child lives ?

Today :	☐0 No ☐1 Yes ☐9 NSP	|__|	SMOKTODAY
From 0-1yr :	☐0 No ☐1 Yes ☐9 NSP	|__|	SMOK01YR
During the pregnancy of the mother :	☐0 No ☐1 Yes ☐9 NSP	|__|	SMOKPREG

8. What type of heating and lighting are used in the rooms where your child lives ?

Heating and lighting by charcoal :	☐0 No ☐1 Yes ☐9 NSP	|__|	CHELCHAR
Heating and lighting by wood :	☐0 No ☐1 Yes ☐9 NSP	|__|	CHELWOO
Lighting by candle :	☐0 No ☐1 Yes ☐9 NSP	|__|	LCAND
Lighting by petrol lamp :	☐0 No ☐1 Yes ☐9 NSP	|__|	LLAMP
Lighting by flash light :	☐0 No ☐1 Yes ☐9 NSP	|__|	LTORCH
Lighting by solar :	☐0 No ☐1 Yes ☐9 NSP	|__|	LSOLAR
Other types of heating and lighting:	☐0 No ☐1 Yes ☐9 NSP	|__|	OTHHEL
If other, define :.....		.....	NAMEOTHHEL

9. Which of the following products are used or stocked in the rooms where you child lives ?

Insecticide (type Yotox, spirales, etc.) :	☐0 No ☐1 Yes ☐9 NSP	|__|	INSECTIC
Deodorants (aerosols) :	☐0 No ☐1 Yes ☐9 NSP	|__|	DEODORA
Incense :	☐0 No ☐1 Yes ☐9 NSP	|__|	INCENSE
Detergents (type Cotol, etc.) :	☐0 No ☐1 Yes ☐9 NSP	|__|	DETERGEN
Petrol, diesel :	☐0 No ☐1 Yes ☐9 NSP	|__|	PETROL
Other types of products :	☐0 No ☐1 Yes ☐9 NSP	|__|	OTHPROD
If other, define :.....		.....	NAMEOTHPR

Diet :

1. Has your child had **diarrhoea without fever** or abdominal pains (colic)

following introduction of non-maternal milk in his diet (cow or goat's milk, milk powder) :	☐0 No ☐1 Yes ☐9 NSP	|__|	DIARINT
after a few months of consuming **non-maternal** (cow or goat's milk, milk powder) :	☐0 No ☐1 Yes ☐9 NSP	|__|	DIARMONTH

2. Currently, how many times, on average, does your child eat the following aliments ?

The consumption of certain aliments is seasonal.

Meat :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMEAT
Fish :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSFISH
Egg :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSEGG
Milk (liquid, powder, curdled) :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMILK
Banana :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSBANA
Mango :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMANG
Melon :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMELON

Orange, lime :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSORAN
Potatoes, sweet potatoes :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSPOT
Vegetables :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSVEG
Millet :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMIL
Sorghum :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSSORG
Maize :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSMAIS
Rice :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSRICE
Wheat (bread, pasta) :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSWHEA
Nuts (Cashew, ground nut) :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSNUT
Prawns, dried oysters :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSPRAWN
Flavouring cubes Maggi :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	CONSCUBE
Other :	☐1 Never ☐2 <1times/week ☐3 1-2 times/week ☐4 ≥1times/day	|__|	OTHALCON
	If other, define :.....	.....	NAMEOTHAL

HISTORICAL SYMPTOMATOLOGY OF ALLERGIC REACTIONS

Asthma :

1. Has a doctor or nurse **already** said that you child has asthma ?

☐0 No ☐1 Yes ☐9 NSP — \|__\| ASTHMA

2. Has your child already breathed noisily or had whistling in his chest whilst breathing

☐0 No ☐1 Yes ☐9 NSP

If **No**, go directly to question **6**

\|__\| WHISTLING

3. During his first two years of life, has your child already breathed noisily or had whistling in his chest whilst breathing ?

☐0 No ☐1 Yes ☐9 NSP — \|__\| WHISTL2YR

If **No**, go directly to question **6**

If **Yes**, how many times (before 2 years of age) ?

☐1 1time ☐2 2times ☐3 ≥3times ☐9 NSP — \|__\| NBWHIS2YR

Between the last two **ramadans**, has your child already breathed noisily or had whistling in his chest whilst breathing ?

☐0 No ☐1 Yes ☐9 NSP — \|__\| WHISTL2RA

If **No**, go directly to question **5**

If **Yes**, at which moment of the year ?

Rainy season :	☐0 No ☐1 Yes ☐9 NSP	|__| WHISTLRS
Dry season :	☐0 No ☐1 Yes ☐9 NSP	|__| WHISTLDS
Harvest time :	☐0 No ☐1 Yes ☐9 NSP	|__| WHISTLHT

Has the noisy breathing of your child been such that it has prevented him from talking normally?

☐0 No ☐1 Yes ☐9 NSP — \|__\| PREVTALK

Has your child already had a rasping cough at night that prevents him from sleeping normally ?

☐0 No ☐1 Yes ☐9 NSP — \|__\| TOUSECHE

Rhinitis and allergic conjunctivitis;

1. Has your child **already had** problems of a runny nose, **or** a sensation of a blocked nose, **or** an itchy nose, **or** sneezing, or loss of the sense of smell **for more than a week,**

irrespective of the frequency of these episodes? ☐0 No ☐1 Yes ☐9 NSP	|__|	RHIN1WEEK
2. Has your child **already had** problems of a runny nose, **or** a sensation of a blocked nose, **or** an itchy nose, **or** sneezing, or loss of the sense of smell **more than 5 times in one year,** irrespective of the frequency of these episodes? ☐0 No ☐1 Yes ☐9 NSP	|__|	RHIN5FAN
Between the **last two ramadans**, has your child **already had** problems of a runny nose, **or** a sensation of a blocked nose, **or** an itchy nose, **or** sneezing, or loss of the sense of smell ? ☐0 No ☐1 Yes ☐9 NSP	|__|	RHIN2RAM
If **No**, go to question **4**		
If **Yes**, at what moment of the year ?		
Rainy season : ☐0 No ☐1 Yes ☐9 NSP	|__|	RHINRS
Dry season : ☐0 No ☐1 Yes ☐9 NSP	|__|	RHINDS
Harvest time : ☐0 No ☐1 Yes ☐9 NSP	|__|	RHINHT
3. Has your child **already had** watery eyes, **or** itchy eyes, **or** an allergic limbo-conjonctivitis? ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJALER
If **No**, go directly to question **1** in the section **Eczema**		
Has your child had, between the **last two ramadans**, watery eyes, **or** itchy eyes, **or** an allergic limbo-conjonctivitis? ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJ2RAM
If **No**, go directly to question **5**		
If **Yes**, at what moment of the year ?		
Rainy season : ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJRS
Dry season : ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJDS
Harvest time : ☐0 No ☐1 Yes ☐9 NSP	|__|	CONJHT
Eczéma :		
Has your child **already had** skin problems with dry patches or seeping cracked patches and itching ? ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEMA
If **No**, **the questionnaire has finished.**		
Between the **last two ramadans**, has your child had skin problems with dry patches or seeping cracked patches and itching ?? ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZE2RAM
If **No**, go directly to question **3**		
If **Yes**, at what moment of the year ?		
Rainy season : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEMARS
Dry season : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEMADS
Harvest time : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEMAHT
1. Have these skin problems affected different parts of the body of your child ?		
Scalp : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZESCALP
Face : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEFAC
Around the eyes and ears : ☐0 No ☐1 Yes ☐9 NSP		
Armpits : ☐0 No ☐1 Yes ☐9 NSP	|__|	ECZEEYEEAR

Elbow :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEARMPIT
Hands :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEELBOW
Under the buttocks:	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEHAND
Groin :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEBUTT
Behind the knee :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEGROIN
Feet :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEKNEE
Other part of body :	☐0 No ☐1 Yes ☐9 NSP	|__| ECZEFEET
		|__| ECZEOTH

What age did your child have when these skin problems of dry patches, weeping cracked patches or itching appear for the **first time**?

☐1 < 2yr ☐2 2 - 4 yr ☐3 ≥ 5yr

\|__\| AGECZEMA

Have your child's skin problems ever been sufficiently important to prevent him from sleeping correctly or waking him up during the night ?

☐0 No ☐1 Yes ☐9 NSP

\|__\| IRRECZEM

Comments : Note with reference to which questions these comments apply

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STROBE Statement—checklist of items included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.