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Human Genetic Contribution to the Outcome of Infection with Malaria Parasites

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1. Introduction

The study of the contribution of human genetics to the risk of severe malaria has a long history, with Haldane in the 1950s reporting a major role of the sickle cell mutation (HbS), in the protection against severe disease (Haldane, 1949). Since then, genetic variants of β-globin (HbE: Hutagalung et al., 1999; HbC: Agarwal et al., 2000; HbS: Aidoo et al., 2002), α-globin (Mockenhaupt et al., 2004), Band 3 protein (Foo et al., 1992), HLA (Hill et al., 1991) and several cytokine loci (Tumor Necrosis Factor-alpha: Knight et al., 1999; Interleukin-12: Morahan et al., 2002a; Interferon-alpha receptor-1: Aucan et al., 2003; Interleukin-4: Gyan et al., 2004) have been demonstrated to confer protection to severe malaria. To date, the majority of studies have been case/control association studies, comparing severe malaria to uncomplicated cases. Due to the fact that *Plasmodium falciparum* is the etiological agent of severe malaria, all studies have consequently focussed on this parasite. The other congeneric species and notably *Plasmodium vivax*, can, however, cause severe disease, albeit at a much lower incidence, and warrant an increased research effort (Price et al., 2007).

Here we argue that for infectious diseases in general and for malaria parasites in particular, more attention should be paid to the "biological" course and outcome of infection in addition to severe disease. This is for several reasons: (i) the majority of infections in endemic settings do not cause severe disease. Indeed, severe disease is a collective term that englobes multiple pathologies (including cerebral malaria, severe malaria anaemia, metabolic acidosis, multiple organ failure) that likely involve very different biological pathways and thus should not be analysed as a single phenotype; (ii) the progression from clinical malaria to asymptomatic infection defines the acquisition of clinical immunity and identifying the mechanisms underlying this tipping point is central to the development of disease control methods; (iii) *P. falciparum* is remarkable in that sterilising anti-parasite immunity is never achieved. Although the parasite has a variety of mechanisms enabling this, the human also plays a part. Identifying pathways that "enable" the parasite to persist without elimination will provide insight into this apparent immune defence dysfunction; (iv) the biology of the parasite within the host will be informative as to how the parasite

optimises its exploitation of and subsequent transmission from the human host. The parasite must persist and transmit to mosquitoes in the face of very differing immune environments. The differential impact of human genetics according to the clinical outcome of infection will throw light on how the parasite manages its strategy for survival and reproduction (transmission).

Focussing on infection enables implementation of a family-based study design that controls for population sub-structure and admixture. Such an approach would be impractical for the study of severe disease because of its relative infrequency. Longitudinal family-based studies enable a more detailed real-time analysis of the human response to infection. Thus, as well as controlling for population sub-structure, they can (i) reveal how the same individual responds at different times in his life and thus generate insight into the acquisition of clinical and anti-parasite immunity; (ii) enable incorporation of parasite genetics both with respect to the long-term co-evolutionary trajectory of the host-parasite duo and the short-term impact of intervention (Loucoubar et al., 2011a).

Evidence for a contribution of host genetic factors to mild clinical malaria and biological phenotypes, such as number of clinical episodes, parasite density, immune responses to *P. falciparum* antigens has progressed with the development of increasingly sophisticated techniques. Population level differences in susceptibility to malaria have been observed between sympatric ethnic groups (Modiano et al., 1996) and, at a finer scale, differential phenotypic expression was observed in monozygotic and dizygotic twins; there was greater phenotypic similarity in monozygotic twins, strongly suggesting genetic control as such twins are genetically more similar than dizygotic twins (Jepson et al., 1995). Segregation studies that assess the extent of phenotypic similarity in families demonstrated co-segregation of parasite density and of prevalence of mild malaria in families (Rihet et al., 1998a). Microsatellite typing of family-based cohorts enabled this segregation to be narrowed down to chromosomal regions (Flori et al., 2003; Garcia et al., 1998; Rihet et al., 1998b; Sakuntabhai et al., 2008; Timmann et al., 2007). Candidate gene approaches have also shown association of specific genetic polymorphisms with mild clinical malaria (Kun et al., 2001; Walley et al., 2004; Williams et al., 1996). Emphasis has understandably been placed on clinical malaria and very few studies have considered asymptomatic malaria and only to a limited extent (Flori et al., 2003; Garcia et al., 1998; Mombo et al., 2003; Rihet et al., 1998a, 1998b; Timmann et al., 2007).

This chapter presents a summary of the achievements in the field of genetic analysis to date, the benefits of examining biological parasite phenotypes and the practical aspects of sampling and analysis. Firstly, we discuss in some detail issues concerning phenotype choice, the pros and cons of case/control vs. family based methods, the importance of context-dependency and of taking into account covariates. We then expand upon the utility of heritability analyses and describe the novel methods that should be a requisite for performing a genetic analysis of quantitative malaria parasite phenotypes. We then discuss our own findings using heritability and genome wide analyses that have led us to propose a novel hypothesis concerning the role of allergy in malaria. Finally we outline the future direction that genetic studies should take, most notably concerning the need to develop tools to examine gene-gene and gene-environment interactions.

2. Malaria parasite course and outcome of infection

2.1 Quantitative malaria-related phenotypes

The malaria parasite spp. lifecycles will undoubtedly be known to readers or covered in associated chapters. Here, we place the life-cycle within a perspective useful for human genetic studies. Although differing in the details, different *Plasmodium* spp. broadly share three distinct life cycle parts within the human host: invasion and asexual proliferation within the liver, invasion and asexual proliferation within red blood cells and the production of sexual stages, gametocytes, from a proportion of these asexually proliferating haploid parasites within the red blood cells. These gametocytes are essential for successful transmission to mosquitoes and subsequent infection of new human hosts. The parasite, as with any other sexually reproducing eukaryote, will, to the best of its capacity, have evolved to optimally exploit its host and maximise its reproductive rate through infection of new hosts. In turn, the human is expected to have evolved to minimise the damage caused by the parasite. The course of infection and the outcome of the human-parasite interaction are thus quantifiable by measurement of the density of asexual and sexual circulating parasites and the frequency of clinical episodes.

Placing the in-host biology of the parasite within the context of the clinical outcome of the infection is central to unravelling how human genetics impacts upon the pathophysiology of malaria. The clinical outcome of infection ranges from severe through mild disease to asymptomatic infection. Less well documented is the progression of clinical expression during the course of a single infection. Early treatment, thanks to considerable public health efforts, has now reduced the burden of disease and in study cohorts we do not know whether an individual with mild malaria would have progressed to severe disease if left untreated and/or eventually control but not eliminate the infection, leading to a chronic long-term asymptomatic infection. Thus, our focus is on parasite biological phenotypes in the context of symptomatic or asymptomatic infection outcomes, with no division into mild *vs*. severe disease. Thus, we ask why is there variation in the density of asexual parasite stages that individuals can withstand before becoming symptomatic and once symptomatic, why do only some infections attain very high densities. Transmission is a crucial part of the lifecycle for the parasite and there is good evidence that the parasite has evolved to optimise its transmission to mosquitoes with respect to the host response to infection (Paul et al., 2003). We thus examine the human factors that influence gametocyte production and whether they differ in symptomatic and asymptomatic infections. Some biological phenotypes, most notably those pertaining to the exo-erythrocytic stages, are beyond our current ability to measure in sufficient detail but do warrant increased research effort. Preventing liver stage infection and eliminating latent hypnozoites in relapsing species are clear targets for reducing the prevalence of infection.

Major differences in certain life-cycle aspects do exist amongst the *Plasmodium* spp. infecting man and surprisingly little is known about the biology or the acquired immune response to species other than *P. falciparum*. The major apparent differences include the capacity to form relapsing latent hypnozoite stages that reside in the liver (*P. vivax* and *Plasmodium ovale*), the rate of development of the asexually replicating erythrocytic stages (48hours for *P. falciparum*, *P. vivax* and *P. ovale vs*. 72 hours for *P. malariae*), the capacity for asexual stage parasites to cytoadhere (*P. falciparum*) and the predilection for invading red blood cells of

differing ages (broadly *P. vivax* and *P. ovale* preferentially invade reticulocytes, *P. malariae* mature red blood cells and *P. falciparum* has a more catholic taste). The duration of a single infection varies significantly: *P. malariae* seemingly lasts up to 30 years despite no evidence of the existence of exo-erythrocytic latent stages; *P. vivax* and most probably *P. ovale* have latent hypnozoite stages and thus although a single blood stage infection may be short-lived (less than a year), relapse extends the duration of a single infection; *P. falciparum* infections can last up to 2 years. Thus, whilst the current emphasis on *P. falciparum* has led to the identification of genetic factors controlling certain clinical and biological phenotypes, their relevance to other species may not be certain and there is much to be done with respect to the three non-falciparum species infecting man (Louicharoen et al., 2009). *Plasmodium knowlesi*, although shown to infect man, has yet to be sufficiently studied to be amenable for human genetic study.

2.2 The phenotype problem

Just as the grouped nature of severe disease yields a poorly resolved phenotype, precise definition of mild clinical malaria and biological phenotypes is equally important and yet arguably more difficult. Defining what is a clinical episode in an endemic setting where malaria co-exists with sundry other infectious diseases is, for the most part, rather *ad hoc*. There are various statistical methods that attempt to define the proportion of fevers attributable to malaria (Smith et al., 1994), but at an individual level body temperature, symptoms associated with malaria and the presence of parasites define a clinical episode. In more studied populations a threshold of parasite density is used in an attempt to account for the high prevalence of asymptomatic infections and the similarity of malaria symptoms with those of other diseases. In practice, local clinicians tend to know when a clinical presentation is a clinical malaria episode but attempts to quantify this and determine quantifiable measurable criteria lead to highly variable definitions. Indeed, within site variation in symptoms and threshold densities will exist not only according to age, but also as a result of human genetics. Biological phenotypes are as complex to define. Although we can measure, for example, asexual and sexual stage parasite densities, the extent to which such data represent any meaningful measure of the host-parasite interaction needs to be considered. Asexual parasite density can alter rapidly and this is especially the case for *P. falciparum*, which has the capacity to sequester. Sexual stage parasite density will to some extent depend on the asexual parasite density, but the added significance of variation in gametocyte density rather than simply gametocyte positivity for transmission to mosquitoes is debatable (Paul et al., 2007). Moreover, at each step (exo-erythrocytic, asexual erythrocytic and sexual erythrocytic), there will likely be variation among parasite clones in the timing and extent of progression through the life-cycle. Specifically, the pre-patent and the asexual growth periods prior to the production of sexual stages will vary among clones. If timing varies so will the densities of parasite stages. On top of this parasite-specific variation, there will be variation resulting from the host-parasite interaction. This will reflect the extent of parasite-specific immunity developed by the host, the general "condition" of the host and "fixed" host genetic factors. Implicit within such host influence is the notion that the phenotypic expression of human genetic variation impacting upon the parasite will vary for an individual depending on that individual's age and history. Independently of any exposure to the specific parasite species in question, an individual's immune response matures over time and can be shaped by exposure (or lack thereof) to non-infectious agents.

Allergy is the paradigm of such immune system maturation. The maturation of the immune system, both innate and acquired, will in turn impact upon the influence of genetic factors that potentially confer protection to malaria parasites. In short, we advocate taking repeated measures from the same individual, as a single snap-shot will not only miss any variability due to the parasite, but also provide no context within which to characterise the individual, beyond obvious factors such as age and gender.

Despite all this noise and natural variation, however, the genetic signal is seemingly strong enough to be detected for many of malaria-related phenotypes. Fine-tuning of the phenotypes may generate more power in more detailed genetic analyses, and one of the simplest methods to assess the strength of the fine-tuning is to perform heritability analyses (see section 4).

2.3 Environmental influence and context-dependency

Acquired immunity is a major factor determining the outcome of an infection. The epidemiology of *P. falciparum* is characterised by premunition and the slow development of acquired immunity. In areas of very intense transmission, there is a relatively rapid development of premunition, whereby the individual can tolerate the presence of the parasite without expressing symptoms. Such clinical immunity thus generates a sub-population who are infected but asymptomatic. As the intensity of transmission decreases, the degree of exposure and age at which premunition develops is progressively later until in areas with low transmission intensity every infection leads to symptoms. The acquisition of immunity that clears the parasite is rarely achieved and only in regions of very intense transmission in old age groups. Acquired clinical and anti-parasite immunity is short-lived and leave of absence of an individual from an endemic area will decrease what little immunity had developed. Thus, human mobility at an individual level is an important confounding factor. In addition, in many areas malaria transmission is seasonal. This seasonal absence/reduction of infectious bites is akin to a period of absence from exposure and can alter the state of the individual and how they respond to an infection in the transmission and "non"-transmission seasons. The epidemiology of *P. falciparum* malaria varies according to the number of infectious bites an individual receives per unit time; importantly, although obvious, the same number of infectious bites spread over two months *vs.* over a year will not yield the same epidemiological profile. The temporal heterogeneity in exposure is a key confounding factor for phenotypic analysis. This will of course be the case for the other species to some extent, but the long duration of *P. malariae* infection and the capacity to relapse for *P. vivax*/*P. ovale* will uncouple the tight link between infectious mosquito bites and the prevalence of infection observed for *P. falciparum*.

A second highly important and often neglected context-dependency is the impact of other co-circulating infectious pathogens. It is widely recognised that multiple co-infecting *Plasmodium* spp. affect each other (Bruce et al., 2000) and the debate over the importance of helminth infections for malaria remains unresolved (Nacher et al., 2000; Spiegel et al., 2003). Whilst concomitant infections can be accounted for, the long term impact of *Plasmodium* spp. on each other is an entirely different problem. There is increasing evidence that there is cross-immunity among *Plasmodium* spp. and accounting for this requires longitudinal sampling. Whilst birth cohorts would be optimal, the investment is considerable. As a

proxy, the development of serological methods that could stratify populations according to level of exposure to all co-circulating would provide a useful tool to examine the long term effects of infection by the community of pathogens on the pathogen of interest.

3. Study populations – Who and how many

A major requisite in any epidemiological study design is defining the sample size that can give the power to detect the effect of interest. For genetic studies, the response traditionally given is "as many as possible" and generally Genome Wide Association studies (GWAS) aim for sample sizes in the thousands. Sample size requirements impose a huge burden and constraint on research and for genetic studies, it is customary that the identified candidate genes are confirmed in a replicate study. The cost of such an endeavour is prohibitive and available to very few laboratories world-wide. Moreover, such large numbers will necessarily include populations from different environments and thus be immediately confounded. For complex diseases, such as malaria, single large effect genes are few and far between. Detecting small effect genes will require a large sample size, but reducing the stringency of the acceptance threshold for candidate gene nomination should be considered. This is especially true if the emphasis shifts from finding the gene, as in monogenic diseases, to identifying important biological pathways. There are multiple solutions to increasing resistance to parasites and repetitive identification of genes involved in specific biological pathways offers convergence towards understanding what governs the outcome of infection.

3.1 Replication

Genetic studies require that candidate genes are confirmed in separate populations. Replication is, however, frequently difficult and causal polymorphisms often have a low effect, increasing, for example, the risk of developing the disease by less than 5-10% (Wu et al., 2010). Moreover, whilst the assumption that there are a few key genes resulting in pathology for non-infectious diseases may be justifiable, this may not be the case for infectious diseases. Malaria is a good example where selective pressure on different populations has occurred relatively recently and thus different ethnicities have evolved different protective mechanisms. Replicating single genetic candidate polymorphisms may not therefore be an entirely appropriate approach and when performed should consider the ethnicities of the study populations. The focus should therefore be placed on the functional consequences of a mutation during an infection and this with reference to the biology of the pathogen and the normal host response. Malaria, on the face of it, is a prime example of this. Sickle cell trait confers protection and yet there are numerous other haemoglobin mutations selected in different ethnicities that potentially offer the same protective solution but via different mechanisms. Whilst some mutations may well exert their protective effect through the same mechanism (e.g. HbC), others may not. The co-occurrence of multiple putatively protective mutations introduces considerable analytical complexity that demands more rigorous consideration than has hitherto been enacted. Furthermore, the emphasis is necessarily shifting away from a pure candidate gene approach to one that considers all the single nucleotide polymorphisms (SNPs) at a locus of interest and focuses on the biological consequences of the mutation.

3.2 Family-based versus case control

Association studies allow identification of genes and their allelic variants involved in susceptibility to disease. They are indispensable for identifying susceptibility genes after candidate chromosomal regions have been revealed by genetic linkage study. The basic method of study compares the allele frequency of a genetic marker from affected (i.e. expressing phenotype) individuals and unaffected control individuals (case-control studies), chosen randomly from a population. The marker used may be a polymorphism without causal relationship to the phenotype or a mutation in a gene candidate. A positive result suggests that the marker studied is involved either directly or by virtue of being linked to the causal gene (i.e. the marker is in linkage disequilibrium with the causal gene whereby marker and causal alleles co-occur more frequently than they would by chance). The major problem with case-control studies is the possibility of false positive results due to differences in environmental factors that influence the development or the evolution of the phenotype being studied. The choice of the control population is one of the most important problems of case-control study: if the control group are not from the same population as the affected individuals, uncontrolled environmental factors or population stratification might induce false positive association. Family-based studies not only account for population stratification, but also increase environmental homogeneity. Classically the major advantage of case-control studies over family-based designs has concerned power. All individuals in case/control studies are unrelated and are thus independent data points, whereas families include individuals who are related and not independent. The non-independent nature of phenotypic data from related individuals can, in fact, be accounted for, as will be discussed in the next section. Moreover, not only is this received wisdom of contrasting power not likely to be as general as believed (Knight & Camp, 2011), but also improved sequencing technology will likely increase the power of family-based designs (Ott et al., 2011). The major limitation of family-based designs remains the identification of sufficient numbers of affected individuals *per se*. Although this may be a problem for extreme phenotypes (e.g. cerebral malaria), it is not for mild malaria and biological phenotypes. Finally, family-based designs offer the possibility of repeated measures, thereby providing a more detailed and complete picture of how an individual responds to an infection. The downside of such longitudinal studies, other than the cost, is the impact that increased access to treatment will have and the potential bias of studying a well-treated population.

4. Preliminary genetic analyses – Heritability

4.1 Application to natural populations

Heritability is an important parameter that indicates the genetic contribution underlying an observed phenotype and provides an indication of the power to detect the effect of individual genes when performing GWAS. A large heritability implies a strong correlation between phenotype and genotype, so that loci with an effect on the phenotype can be more easily detected (Visscher et al., 2008). Estimation of heritability in its broad sense in natural populations is not possible and hence narrow sense heritability, which estimates the additive genetic contribution, is calculated. Actual values of heritability are specific for a study population at a particular time and thus not strictly comparable among studies, although broad trends can be inferred.

Heritability analyses have until recently remained the quantitative tool of animal and plant breeders. They have been relatively ignored by human geneticists and the study of natural populations for several reasons. Firstly, to generate sufficient data, well-conducted longitudinal family-based epidemiological studies that take into account confounding environment factors are required (Ntoumi et al., 2007). This requires a considerable investment. Secondly, because the genetic component is not measured directly but is inferred from the resemblance between relatives and because relatives often live in the same house, differentiating genetic from the shared environment is problematical. Inadvertent exclusion of a key environmental factor would erroneously lead to substantial over-estimates of heritability. Thirdly, the statistical methods that can manage repeated measures inherent in longitudinal surveys for robust heritability analyses have only recently been developed. Finally, given the relative inaccuracy of heritability estimates and the increasing ease with which genome wide analyses can be performed, the added value of calculating heritability has been considered questionable.

This view of the utility of heritability analyses has been largely colored by its extensive historical use in breeding programs where projections of selection experiments are invaluable. For the study of complex infectious diseases, the value of heritability lies elsewhere and goes beyond the simple question of evaluating the potential genetic contribution to a phenotype. This "novel" value of heritability is well exemplified by the recent observation that there is considerable missing heritability in GWAS of more complex diseases (Manolio et al., 2009); only a fraction of estimated heritability can be accounted for by the genes identified in GWAS. Without initial estimation of heritability, this anomaly would not have been identified. The potential causes for this, include potentially important roles of epistasis, gene-environmental interaction and the confounding effect of population specific genetic architecture (Eichler et al., 2010). In addition to genetic explanations, one potential source contributing to the missing heritability concerns the phenotype; poorly resolved phenotypes lower the power to detect genetic variants (van der Sluis et al., 2010). One important point often misunderstood is that the absence of heritability of a phenotype implies no genetic contribution – this is not true. Narrow sense heritability measures the proportion of the variance in the phenotype explained only by additive genetics and there can be non-additive genetic effects. Furthermore, a causal variant that has an effect on a phenotype, but which is present at 100% allelic frequency will have zero heritability. Conversely, a large heritability does not imply that only a few genes are involved.

4.2 Repeated measures, complex pedigrees and statistical analyses

Historically, heritability analyses have used single measures of the phenotype or a summary variable when repeated measures were performed. Such summary measures tend to lead to over-inflated estimates of heritability and in the advent of available statistical methods, should be avoided. Likewise, heritability analyses used to analyse the residual of the phenotype after having taken into account other covariates. Such an approach assumes that there is no interaction between the genetic factors and these other covariates, an assumption that is likely to be invalid. Statistical methods now exist whereby simultaneous analysis of the genetic and environmental contribution to a phenotype is possible.

Heritability analyses of phenotypes gathered through repeated measurements of individuals from a community with a complex pedigree structure must take into account the

bias introduced by multiple measures from the same individual and the fact that individuals are related. The individual observations are not independent. Although taking a single measure for an individual can overcome the first issue, multiple measures from the same individual are informative as they provide a notion of the repeatability of the phenotype and enables calculation of the intra-individual or permanent environment effect. This intra-individual variation contains features that are particular to each individual. This will include house effects, maternal effects, individual behaviour and non-additive genetic effects. The house and maternal effect can be taken into account by using appropriate matrices; each pair of individuals either do (1) or do not (0) share the same house or mother.

Creating a genetic relatedness matrix of the study population is not only central for heritability calculations, but extremely useful to take into account the non-independence of individuals when performing classical regression analyses. The genetic covariance (the familial relationship) among all pairs of individuals in the study cohort can be simply calculated using the pedigree information as follows:

For A and B, a given pair in a pedigree, the genetic covariance is computed as r(A,B) = 2x coancestry(A,B) where the coancestry between A and B is calculated using the method presented in 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 IX is the inbreeding coefficient of an individual X, which is equal to the coancestry between the two parents of X. IX is set to 0 if X is a founder. The consequent Pedigree-based genetic relatedness matrix has dimensions KxK, where K is the total number of individuals in the pedigree including those with missing phenotypes. This matrix can be built using INBREED procedure of SAS. A house matrix can also be constructed whereby a value of 1 is ascribed if the relative pair shares the same house or 0 otherwise. Likewise, to examine the extent that there are maternal effects that are passed onto offspring, a maternal matrix can be established.

Repeated measures analyses are best handled using Generalized Linear Mixed Models (GLMM). Mixed models enable fitting of random effects. Random effects are assumed to be normally distributed, and conditional on these random effects, data can have any distribution in the exponential family (e.g. Gaussian, Binomial, or Poisson). For repeated measures of unrelated individuals the random variable would simply be the individual identity. For related individuals, the genetic relatedness matrix will take into account the individual repeated measures plus the bias introduced by the non-independence of observations from related individuals.

Heritability analyses seek to decompose the total variance of the phenotype in question into components explained by additive genetic, intra-individual, house effects. Heritability is the proportion of the phenotypic variance that is due to additive genetics. Other covariates, such as age, gender etc, can also be taken into account. Although there are several programs able to perform such analyses, we have found that SAS offers a complete and yet flexible library of procedures (version 9.1.3, SAS Institute Inc., Cary, NC, USA), notably GLIMMIX, MIXED and INBREED. For count outcome variables (e.g. parasite density, number of clinical episodes per unit time), a Poisson regression model is fitted, which explicitly takes into account the non-negative integer-valued aspect of the dependent count variable. Therefore a GLMM with a Poisson distribution can be fitted using GLIMMIX and *log* as the

link function between E(*variable* | *covariates*). For binary outcome variables (e.g. presence or absence of gametocytes), GLMM are fitted with a Binomial distribution with a *logit* link function. A maximal model with all covariates is fitted and a minimal adequate model including only significant covariates obtained. The effect of each covariate on the outcome variable is estimated taking into account inbreeding, via the genetic relatedness matrix integrated in GLIMMIX using the LDATA option, repeated measures and house effects.

5. Heritability analyses of malaria phenotypes in longitudinal family-based cohorts

5.1 Study sites and populations

Our study sites include three family-based cohorts that have been followed for over ten years. Two of the cohorts occur in Senegal but differ by an order of magnitude in transmission intensity; in addition to *P. falciparum*, both *P. malariae* and *P. ovale* co-circulate. The third cohort is a large Karen community in Thailand with low transmission intensity but equal incidence of *P. vivax* and *P. falciparum*.

The Dielmo and Ndiop longitudinal surveys in Senegal have been described in detail elsewhere (Rogier et al., 1999; Sakuntabhai et al., 2008; Trape et al., 1994). Briefly, a longitudinal cohort study of malaria has been carried out since 1990 in Dielmo and 1993 in Ndiop. In Dielmo there are 594 individuals forming 190 nuclear families and in Ndiop 653 in 208 nuclear families. In each cohort, the majority of individuals form one large complex family: 453 individuals in Dielmo and 503 in Ndiop. Overall there are 10 completely independent families in Dielmo and 21 in Ndiop. The ethnic composition differs between the two cohorts. In Dielmo, the ethnic groups consisted of 79% Serere, 11% Mandinka and 10% miscellaneous, whereas in Ndiop, there were 76% Wolof, 19% Fulani and 5% miscellaneous. Family structures were constructed by using a questionnaire, interviewing each individual or key representatives of the household to obtain both demographic information such as birth date, age, sex and genetic relationships between children, their parents, and sometimes their grandparents or non-relatives in the same household, and other households. Previous typing with microsatellites enabled the construction of a pedigree based on Identity-by-Descent (IBD) using MERLIN (Abecasis et al., 2001; Sakuntabhai et al., 2008), thereby enabling constitution of the genetic relatedness matrix.

Malaria transmission is perennial in Dielmo, where a river maintains larval breeding sites for the mosquitoes even in the dry season. The number of infective bites per person per year (Entomological Inoculation Rate, EIR) is of the order of 200. By contrast, malaria transmission is strictly seasonal in Ndiop and dependent upon the rainy season that occurs from July-September and the EIR is approximately 20. Such differing transmission has marked consequences on the epidemiology of malaria in the villages. This is most evident in the higher *P. falciparum* prevalence rates of infection in Dielmo (80%) compared to the seasonal rates in Ndiop that change from 20% in the dry season to 70% in the rainy season (Sakuntabhai et al., 2008). Peak incidence of clinical *P. falciparum* infections occurs in the 1-4 year old age group in Dielmo and in the 8-12 year old age group in Ndiop.

In Thailand, a community-based cohort study has been on-going since 1998 (Phimpraphi et al., 2008a), situated in a mountainous area of Ratchaburi province, Thailand near the Thai-Myanmar border. Suan Phung has a total population of 5,368 living in 7 hamlets. The ethnic

composition of this community is majoritarily Karen (85%), with Thai (14%) and the rest are Mon and Burmese (1%). The total pedigrees are comprised of 2,427 individuals, including absent or deceased relatives. There are 238 independent families containing 603 nuclear families; the majority are 2 generation-families with family size range from 3 to 958. The epidemiology of malaria has been described elsewhere (Phimpraphi et al., 2008a). Briefly, the incidence of malaria is highly seasonal with annual peaks in May-June. Incidence was low, peaking at 141 episodes of *P. falciparum* per 1000 person-years and 70 for *P. vivax* over the 6-year intense study period. In this site, virtually all infections lead to febrile episodes and thus there is no information on asymptomatic infections. Peak incidence occurs in an earlier age group (5-9 years old) for *P. vivax* than for *P. falciparum* (10-15 years old). Parasite densities of either species peak in the <10 years old age group. Microsatellite genotyping again enabled the construction of a pedigree based on IBD (Phimpraphi et al., 2008b).

5.2 Heritability of malaria-related phenotypes with differing transmission intensity

Heritability analyses were conducted on several clinical and biological malaria-related phenotypes. The major non-genetic factors included age and variables concerned with differential extent of exposure on both a temporal (season, year) and spatial scale (hamlet, house). Figure 1 summarises the differential impact of the non-genetic and genetic factors on the number of clinical episodes (*P. falciparum* and *P. vivax*), the asexual parasite density

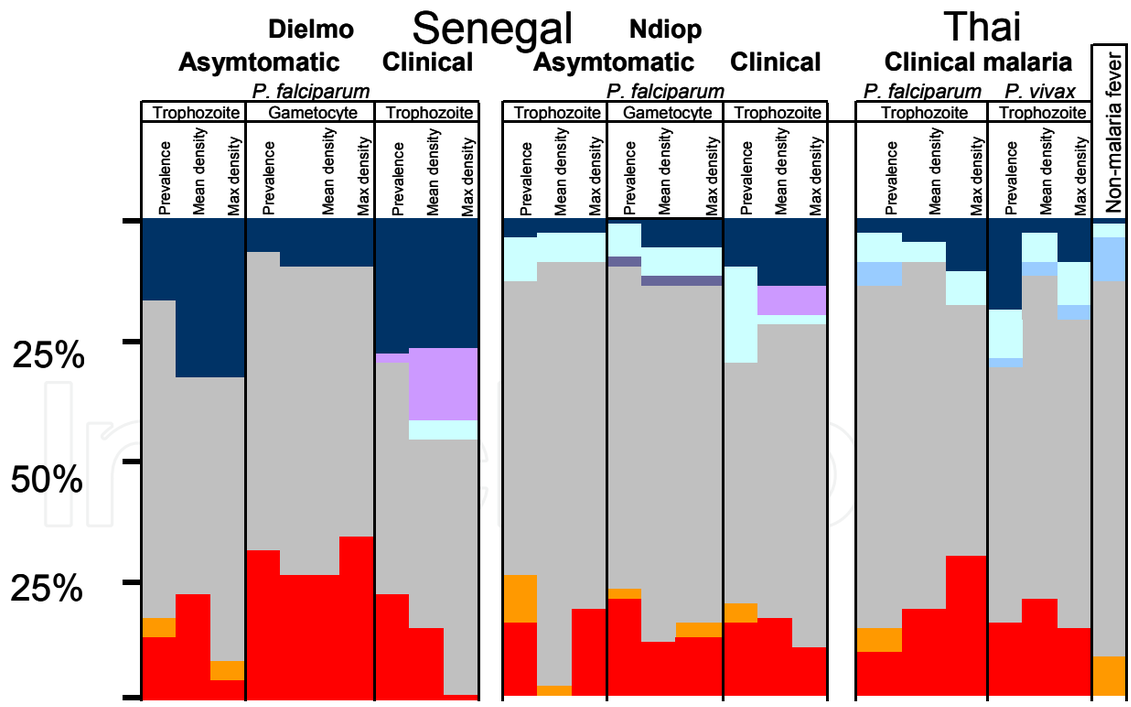


Fig. 1. Percentage of variance in malaria-related phenotypes explained by additive genetics (red), house (orange), hamlet (light blue), age (dark blue), date (turquoise), days in village (mauve), asexual parasite density (dark grey) and unknown (light grey). Malaria-related phenotypes include the prevalence of asymptomatic and clinical episodes and the mean and maximum asexual parasite density (trophozoite) during asymptomatic and clinical episodes.

during both clinical and asymptomatic infections and gametocyte prevalence and density, but only during asymptomatic infections; there was no significant heritability for gametocyte phenotypes during symptomatic infections. Non-malaria fever is included in the Thai study despite an absence of any genetic contribution, because of its significant correlation with malaria phenotypes.

5.2.1 Impact of non-genetic factors

Overall, it can be seen that age is more important in the highest transmission cohort (Dielmo) and decreases in importance as transmission intensity drops. This reflects the acquisition of clinical and anti-parasite immunity with exposure. A finer measure of exposure is given by the number of days present in the cohort, which has a significant impact on the number of clinical episodes. This effect not only reflects the probability of being infected, but also the well-recognised short-lived nature of clinical immunity: despite a history of exposure, absence from a malaria endemic setting can reduce the extent of clinical immunity developed. Temporal variation in the number of clinical episodes and in parasite density during both clinical and asymptomatic parasite density is notable for the Senegal cohort of highly seasonal transmission (Ndiop). Whilst seasonal variation in clinical episodes is expected, a seasonal effect on parasite density is less easily explained, especially for asymptomatic infections. This same effect is present for the production of gametocytes and we have recently shown that variation in mosquito biting rate has an impact on both asexual parasite density and gametocyte production (unpubl. data). This effect is seemingly linked to immune status of the individual, notably the levels of IgE.

Spatial variation is evident among the seven hamlets in the Thai site, but has little impact at the finer scale of house. In zones of high transmission intensity, where there is effectively saturating transmission and thus homogeneous exposure, fine scale spatial differences may not exist. The relatively small effect of house, even at lower transmission intensities, may be the result of the biting behaviour of the mosquitoes. In the Thai study site, the two mosquito vector species are *Anopheles minimus* and *Anopheles maculatus*, which are predominantly exophilic, thereby explaining the lack of a house level effect (Somboon et al., 1994). In Ndiop, Senegal, the major vector is *Anopheles arabiensis*, which will bite both indoors and outdoors and will also bite cattle. This non-specialist host choice may result in a biting distribution that is less house-oriented, being also affected by the distribution of animal enclosures. Although the occurrence of a small house effect in all three sites for several phenotypes, including parasite density, might reflect very local scale variability in biting rate, the confounded relationship of genetic relatedness and house may not be sufficiently resolved. Analysis of the heritability in the Thai study site with and without taking into account house led to estimates of the genetic contribution of 12% (with house) *vs*. 20% (without house) for the number of clinical episodes. The absence of any house effect for clinical *P. vivax* cases but the presence of such an effect for *P. falciparum* in the Thai site is likely because of relapse rather than differential biting habits of different mosquito vectors, although there may be differential transmission of the two species by different mosquito spp. (Somboon et al., 1994). There is no analytical approach to enable complete differentiation of the genetic and share house effects, especially in the case where there are no relatives in the cohort living in different houses. In our case, the cohorts all contain relatives living in different houses and non-relatives sharing households and thus the

genetic relatedness and house matrices will enable both effects to be estimated, even though they are clearly confounded. Parasite density phenotypes during clinical episodes were not influenced by house in any site, consistent with the hypothesis that although house can contribute to the tendency to become infected, it does not impact upon the parasite once the infection has started.

5.2.2 Impact of genetic factors

Examination of the genetic contribution among the study sites reveals evidence for a strong genetic contribution to the number of *P. falciparum* clinical attacks: 12%, 16% and 22% with increasing transmission intensity. Interestingly, excluding household effects, results in contributions of approximately 20% in all 3 sites. The similarity of these values is remarkable given the very differing transmission intensities observed and the differing ethnicities. The marginal decrease in heritability with the decrease in transmission intensity likely reflects the increased heterogeneity in exposure at lower transmission intensities. The difference in the contribution of genetics is, however, small given the very large difference in transmission intensity. Moreover, the heritability of the number of clinical episodes is quite conserved irrespective of the phenotypic definitions used, the geographical region and even the *Plasmodium* species implicated. Similar values were observed in a study in Kenya (20%; Mackinnon et al., 2005) and a study in Sri Lanka (15%; Mackinnon et al., 2000) and for *P. vivax* (19% in Thailand (Phimpraphi et al., 2008b) and 15% in Sri Lanka).

A striking feature is the extensive differences in the impact of genetics on the mean *vs*. maximum parasite density. Parasite density has been recognised for a long time to be under human genetic control (Garcia et al., 1998; Rihet et al., 1998a), but the biological meaning of measures of parasite density requires contextual interpretation. The maximum parasite densities in both clinical and asymptomatic infections clearly indicate the extent to which the parasite can be controlled during and prior to the onset of a clinical episode. However, whilst the latter intuitively pinpoints the density at which an individual will clinically express the infection, a run-away symptomatic infection may attain exaggerated densities that are poorly informative of the biological pathways involved. The mean parasite density provides at least some replicated measure of how the individual controls a single infection, being less reliant on the extreme values. Although inherited blood disorders might result in generally lower parasite densities, the major pathways influencing parasite density will certainly be those of the immune system. Age has often been used as a proxy for the acquisition of immunity and accounting for age should reduce the contribution of that arm of the immune system to the genetic effect. It should be noted, however, that there may be a genetic contribution to acquired immunity (Stirnadel et al., 1999) and there is now some evidence that the inherited blood disorders operate interactively with the acquired immune response (Amaratunga et al., 2011). Nevertheless, our analyses would suggest that there is a contribution to the genetic effect from the innate arm of the immune system.

Gametocyte carriage has been associated with a worsening blood environment for the parasite (e.g. fever responses, anaemia) (Nacher et al., 2002; Price et al., 1999). However, such cues are only associated with symptomatic episodes of malaria and it is now well established that asymptomatic infections can also generate gametocytes and infect mosquitoes (Bousema & Drakeley, 2011). Differences in the tendency of sympatric ethnic groups to carry gametocytes have been noted for a long time (Perry 1914; Paganotti et al.

2006). We found a significant human genetic contribution associated with gametocyte prevalence in asymptomatic *P. falciparum* infections. By contrast, there was no heritability associated with the production of gametocytes for *P. falciparum* or *P. vivax* symptomatic infections. Increased gametocyte carriage has been observed in individuals with HbC (Gouagna et al., 2010) and HbS, although its contribution was small (Lawaly et al., 2010). Clearly there are other genes implicated in eliciting gametocyte production by the parasite. Thus, a proportion of the population is susceptible to carry gametocytes and be super-spreaders. Targeting such individuals with specific intervention methods is an intriguing option.

5.3 Correlation among phenotypes

Examining the correlations among phenotypes can be extremely informative in characterising the outcome of infection. The number of clinical episodes and maximum parasite density were positively associated with each other in the Senegalese cohorts (Dielmo r=0.54; Ndiop r=0.34) but not in Thailand. The correlation was strongest in the cohort of highest transmission intensity (Dielmo). In Thailand, there was comparatively little variation in the number of clinical episodes experienced by any individual (1-13). The positive correlation between these two phenotypes is consistent with the interpretation that individuals experiencing many *P. falciparum* clinical attacks have a poor capacity to control parasite density, which thus frequently reaches the high threshold density necessary to elicit a clinical attack (Rogier et al., 1996). As immunity develops, the human will be increasingly capable of controlling the proliferation of the parasite and keep the densities at sub-clinical levels. However, there was a negative correlation between the ability to harbour high parasite loads without symptoms and the number of clinical malaria attacks. Our results point to a genetic influence on the control of parasite density governing the occurrence of clinical attacks. In other words, higher asymptomatic parasite loads seemingly protect against occurrence of clinical episodes. One possible mechanism is through maintenance of an efficient concomitant clinical immunity reducing the risk of developing clinical malaria despite the presence of a relatively elevated parasite load. This is the first indication for a genetic basis to premunition acquired by individuals living in endemic areas (Sergent & Parrot, 1935). The immunological basis of such premunition remains elusive. Interestingly, high parasite specific IgE levels in asymptomatic infected individuals have been shown to be reduce the odds of a clinical episode (Bereczky et al., 2004), thus suggesting the Th1/Th2 balance and hence the relative cytophilic IgG1/3 and IgE titres produced in response to parasite density determine the progression to clinical disease.

The Thai analyses demonstrated a strong negative correlation between the number of non-malaria fevers and *P. falciparum* cases, suggesting that illness due to non-malaria pathogens may protect from *P. falciparum* infection or disease and vice versa. That there was no genetic contribution to non-malarial fever would suggest that there is no genetic trade-off generating susceptibility to *P. falciparum* with protection from non-malaria fever. However, the non-malaria fever phenotype is very imprecise and thus absence of a genetic effect may be a phenotype problem. Nevertheless, the negative correlation underlines the importance of considering malaria infections in context of other circulating pathogens. In contrast, there was evidence of between *Plasmodium* spp. interactions. There was a positive correlation between the number of *P. vivax* episodes and maximum *P. falciparum* parasite density,

suggesting that common mechanisms are involved in determining these phenotypes. Individuals previously infected with *P. vivax* during the early years of study (1998-2000) had higher maximum *P. falciparum* parasite densities in 2001-2004. They also had a greater number of *P. vivax* episodes, but did not have significantly different maximum *P. vivax* parasite densities from those not previously infected with *P. vivax*. This suggests that common mechanisms do govern susceptibility to infection by *P. vivax* and control of *P. falciparum* density but not control of *P. vivax* density. Given the differences in red blood cell tropism, although blood disorders may be implicated, the involvement of shared immunological mechanisms is likely, supporting the hypothesis that there is significant cross-species protective immunity (Loucoubar et al., 2011b).

6. Linkage analyses of malaria phenotypes identifies regions associated with atopy

Linkage analyses are the classical statistical genetic analyses testing for co-segregation between a chromosomal region and a phenotype of interest. Prior to the high density genotyping now available for genome wide association, linkage analysis was the preferred method for gene discovery, enabling chromosomal regions of interest to be identified with relatively low genotyping coverage.

6.1 Genome wide linkage analysis of *P. falciparum* in Senegal

Our genome wide linkage analysis in the two Senegalese cohorts identified three novel regions of linkage in addition to the 5q31 region that has been previously reported to be linked to asymptomatic parasite density (Flori et al., 2003; Garcia et al., 1998; Rihet et al., 1998b). All of the regions have been previously found to be linked to asthma/atopic disease or related phenotypes, such as IgE titres (Iyengar et al., 2001; Jang et al., 2005; Kurz et al., 2006; Xu et al., 2001; Zhang et al., 2003). The novel regions of linkage were chromosome 5p15-p13 and 13q13-q22 linked with the number of *P. falciparum* clinical malaria attacks in Dielmo, and chromosome 12q21-q23 with the maximum parasite density during asymptomatic carriage in Ndiop. The linkage results differ for the two cohorts, likely reflecting the important differences both in the ethnic backgrounds and in the prevailing transmission conditions. Interestingly, the 5q31 locus has been linked to several immune related disorders, including asthma/atopy (Meyers et al., 1994), inflammatory bowel disease (Lee et al., 2002), Crohn disease (Peltekova et al., 2004), Celiac disease (Latiano et al., 2007) and psoriasis (Friberg et al., 2006). Moreover, genes within the 5q31 locus have been suggested to regulate delayed-type hypersensitivity responses associated with Leishmania chagasi infection (Jeronimo et al., 2007). This region contains a cluster of cytokines, among which IL12B may play a critical role since it has been associated with some immune-related diseases. An insertion/deletion polymorphism in the promoter region of IL12B has been reported to be associated with psoriasis (Cargill et al., 2007) and cerebral malaria (Morahan et al., 2002a) while two intronic SNPs were associated with asthma (Morahan et al., 2002b; Randolph et al., 2004).

With the exception of the β-globin locus, there was no overlap of the regions of linkage that we detected and the location of the genes that have been previously reported to be associated with severe/cerebral malaria. This apparent discordance between genes

responsible for severe malaria and those controlling the response to *Plasmodium* infection in our study may also indicate that the mechanisms (and genes) involved in the protection against severe malaria are largely independent of those involved in the response to mild clinical malaria and/or the control of blood parasitemia.

6.2 Role of allergy in malaria

The acquisition of immunity to the human lethal malaria parasite *P. falciparum* develops very slowly and is not sterilising. Even in zones where the transmission intensity is high, the development of immunity only results in a premunition leading to a reduction in the number of clinical episodes and the progressive control of parasite density. Cytophilic immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by opsonisation, play an important role in this premunition (Wilson & McGregor, 1973). An important role of the Th1/Th2 balance in the development of clinical malaria has been suggested by numerous studies (e.g. Elghazali et al., 1997). Orientation of the immune response towards a Th1 versus a Th2 profile, will respectively promote IgG *vs.* IgE proliferation. The role of IgE in clinical and severe malaria is still poorly documented and results are controversial. *P. falciparum*-specific IgE is elevated in malaria patients and has been proposed to play a pathogenic role in severe malaria (Elghazali et al., 1997; Perlmann et al., 1997), although high levels in individuals with asymptomatic infections were associated with protection (Bereczky et al., 2004). The induction of a Th2 biased immune response by *P. falciparum* may generate a tendency to develop a Th2 type immune response to other antigens. Dendritic cells that are oriented to a Th2 phenotype by an antigen are more susceptible to orient the immune response towards a Th2 profile when confronted by a second antigen (de Jong et al., 2002). It has been suggested that the Th2 bias induced by *P. falciparum* may exacerbate allergy and explain the higher than normal frequency of several cancers in malaria endemic populations (Taylor-Robinson, 1998).

Direct evidence has been found for a pathogenic role of histamine (a major effector molecule in allergy response) in mouse malaria models using both genetic and pharmacological approaches; histamine binding to Histamine receptor-1 (H1R) and receptor-2 (H2R) increases the susceptibility of mice to infection with *Plasmodium* and histidine decarboxylase–deficient mice, which are free of histamine were highly resistant to severe malaria (Beghdadi et al., 2008). H1R mediates most of the proinflammatory effects of histamine (Bryce et al., 2006). The anti-inflammatory and immunosuppressive effects of histamine are largely dependent on stimulation of H2R. In addition, there is suggestion that histamine might influence the polarization of T-helper cell development through inhibitory effects on dendritic cells (Idzko et al., 2002). Reports indicate that specific components of the innate immune system, including eosinophils (Kurtzhals et al., 1998), basophils (Nyakeriga et al., 2003), and Mast cells (MCs) (Furuta et al., 2006), could play important roles in the pathogenesis of malaria. 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 (Bhattacharya et al., 1988; Srichaikul et al., 1976). Chlorpheniramine, a histamine receptor-1 agonist reversed resistance to chloroquine and amodiquine both in vivo and in vitro (Sowunmi et al., 2007). Moreover, astemizole, another HR1 agonist, was identified as an antimalarial agent in a clinical drug library screen (Chong

et al., 2000). 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 (MacDonald et al., 2001). How this could benefit the parasite is not known, but the vasodilatory effects of histamine might permit the parasites to circulate more readily and histamine might increase endothelial cell-surface expression of thrombomodulin, which is both a tissue anticoagulant and a receptor for parasitized erythrocyte sequestration.

Our heritability, correlation and genome wide linkage study results are consistent with there being a relationship between malaria and allergy and raise the hypothesis that the development of clinical malaria may be due to an allergic reaction to malaria parasites or by-products of parasite infection, or that allergy/atopy and the response to malaria infection may share common mechanisms. Thus, clinical immunity to malaria may indeed be immunotolerance and absence of allergic-type responses rather than the presence of neutralising antibodies to malaria "toxins" as previously suggested (Jakobsen et al., 1995). Several lines of additional evidence support the concept that susceptibility to malaria and atopy may be related to the same immunological defect. In Ethiopia, atopic children had a higher prevalence of malaria attacks (Haileamlak et al., 2005), while in Tanzania maternal malaria had a protective effect on wheezing in children age of four (Sunyer et al., 2001). Finally, a mouse model for human atopic disease (NC/Jic) was found to be susceptible to murine malaria (Ohno et al., 2001) and a major quantitative trait locus (derm1) for atopic disease mapped close to the region controlling parasitemia (Kohara et al., 2001).

7. Genetic association studies and the post-genomic era

Most of the protective variants are thought to have emerged in populations living in regions endemic for malaria as a result of the high selective pressure due to the parasite (Kwiatkowski, 2005). The past decade has seen growing evidence of ethnic differences in susceptibility to malaria and of the diverse genetic adaptations to malaria that have arisen in different populations. The fact that different malaria-resistance alleles have arisen in different places suggests that a great deal of selection by malaria has happened relatively recently in human history and certainly since human migration out of Africa (Eid et al., 2010). Such population differences in susceptibility to malaria are becoming more amenable to study since the development of high through-put genetic technology, thereby allowing us to genetically dissect the outcome of infection.

7.1 Candidate and genome wide association studies

Association studies are used for identifying genes and their common allelic variants involved in predisposition to a disease. Such studies are performed after localization of susceptible loci by linkage analysis. This method compares the allele frequency of a genetic marker of affected and non-affected individuals, chosen at random in a population (case-control study). The marker might be the causal polymorphism or any polymorphism in linkage disequilibrium (LD) with the causal one. A positive association with one marker suggests that this marker is in LD with the causal polymorphism. The LD between two markers is defined by the existence of a combination of alleles of these markers more often than expected by chance. The choice of the control population is one of the most important problems of case-control studies: if the control group is not from the same population as the affected individuals, uncontrolled environmental factors or population stratification might

induce false positive association. Association studies are the most widely used contemporary approach to relate genetic variation to phenotypic diversity. This is due to their higher power and lower cost to detect a susceptibility locus than linkage analysis.

The genome era has heralded unparalleled possibilities to identify genetic variants that underpin disease (http://www.genome.gov/gwastudies). The majority of these initial studies have concerned non-infectious diseases and, for the most part, examined dichotomous disease phenotypes, nominally affected and unaffected. Application to infectious diseases has only been relatively recent (Thye et al., 2010; Zhang et al., 2009) and for malaria has focussed on severe disease (Jallow et al., 2009). Genome wide association studies of clinical malaria and biological phenotypes are currently underway in our laboratory and whilst certain to reveal many novel candidate genes of importance, we know this is not enough. It is widely recognized that common multifactorial diseases are caused by multiple genetic and environmental factors and interactions among all these factors.

7.2 Multiple loci and gene x environment interactions

Following the success of identifying genes underlying diseases resulting from single locus mutations and inherited in a Mendelian fashion, it has become clear that there are many complex diseases that are inherited in a non-Mendelian fashion. That there are many loci affecting a trait is no surprise, but the simultaneous analysis of many loci is problematic for several reasons, especially when searching for novel genes as in the case of GWAS. The first is the curse of dimensionality - there are more candidate loci than there are observations. The second is the extent to which genes exert their effects independently, or whether there exist interactions among genes with respect to the phenotype. Thirdly, there is the question of whether there exists gene x environment interactions.

With the development of genotyping technologies, GWAS have become the method of choice to identify complex disease associated genes using SNPs as biomarkers (Hardy & Singleton, 2009). In the past three years, about 400 GWAS have successfully identified more than 531 genetic variants associated with various traits or diseases (Manolio et al., 2009). Standard analytical approaches in GWAS have proceeded by individually testing each SNP of the hundreds of thousands of genotyped SNPs. Thus, only SNPs that have a relatively strong marginal effect have been detected, explaining only a small part of the heritability. Hence, other SNPs that have weaker association with disease and/or act primarily through a complex mechanism involving interactions with other genetic variants and environmental factors have yet to be discovered. There is an increasing number of statistical methods and software that are being proposed to allow analysing multiple genetic markers and their interactions simultaneously (Cordell, 2009), but the identification of these interactions remain very challenging. Powerful methods for conducting genome-wide interactions studies are therefore needed. One of the interesting features of GWAS is that the same loci were found associated with several diseases (e.g. cancers, cardio-vascular diseases, auto-immune diseases), suggesting that genes with a pleiotropic effect may be more frequent than anticipated and may play a key role in basic physiopathological mechanisms underlying a number of diseases. The identification of pleiotropic genes which are likely to influence master regulators of biological processes is therefore of major importance. Studying together diseases that are supposed to share common genetic determinants can facilitate the characterization of such genes.

8. Conclusion

The ultimate goal of studying the human genetics underlying infectious disease is to identify key biological pathways that determine the outcome of infection. Human genetics studies of malaria have, to date, almost exclusively focussed on severe disease caused by *P. falciparum* and yet this is a relatively rare event that regroups many pathologies. The majority of infections cause mild clinical symptoms or are asymptomatic. Asymptomatic infections are evidence for the development of clinical immunity (premunition), acquired at a rate proportional to exposure. Quantifying the frequency of a symptomatic *vs*. an asymptomatic outcome of infection and the parasite densities during such outcomes provides a measure of the host-parasite interaction. More detailed parasite phenotypes, such as those looking at particular parasite stages, provide additional insight into how the human response to infection determines the phenotype. Longitudinal family-based cohort studies offer the opportunity to generate non-severe malaria-related phenotypes for individuals over time, allowing the progression towards premunition to be examined. Family-based studies offer the possibility of assessing the robustness of phenotypes through simple heritability analysis. Fine-tuning phenotypes as such will save time and money during the human genotyping stage and subsequent analysis. Our cohort studies revealed surprisingly similar human genetic contributions to the clinical outcome of infection despite very large differences in transmission intensity. Identifying and accounting for confounding factors and covariates is essential and the similarity of the heritability values is reassuring for the robustness of the phenotype and implicitly suggest that covariates have been correctly accounted for. However, the potential that such covariates interact with human genes of interest should not be forgotten and future focus on gene-environment interactions is paramount. In addition, we identified for the first time a human genetic contribution to the transmission of the parasite. This opens up exciting possibilities for targeting transmission as well as disease. From our subsequent linkage studies, we found evidence that similar biological pathways govern the clinical outcome of a *P. falciparum* infection and allergy. In addition to the potential therapeutic possibilities, that common biological pathways govern multiple diseases offers the huge potential for combining efforts across multiple domains.

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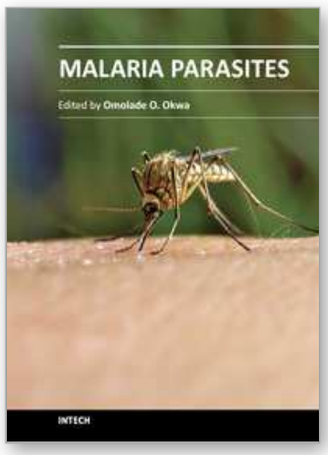
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Malaria Parasites

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Malaria is a global disease in the world today but most common in the poorest countries of the world, with 90% of deaths occurring in sub-Saharan Africa. This book provides information on global efforts made by scientist which cuts across the continents of the world. Concerted efforts such as symbiont based malaria control; new applications in avian malaria studies; development of humanized mice to study P.falciparium (the most virulent species of malaria parasite); and current issues in laboratory diagnosis will support the prompt treatment of malaria. Research is ultimately gaining more grounds in the quest to provide vaccine for the prevention of malaria. The book features research aimed to bring a lasting solution to the malaria problem and what we should be doing now to face malaria, which is definitely useful for health policies in the twenty first century.

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