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Application of Genomics to Field Investigations of Malaria by the International Centers for Excellence in Malaria Research

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Abstract

Success of the global research agenda toward eradication of malaria will depend on development of new tools, including drugs, vaccines, insecticides and diagnostics. Genomic information, now available for the malaria parasites, their mosquito vectors, and human host, can be leveraged to both develop these tools and monitor their effectiveness. Although knowledge of genomic sequences for the malaria parasites, *Plasmodium falciparum* and *P. vivax*, have helped advance our understanding of malaria biology, simply knowing this sequence information has not yielded a plethora of new interventions to reduce the burden of malaria. Here we review and provide specific examples of how genomic information has increased our knowledge of parasite biology, focusing on *P. falciparum* malaria. We then discuss how population genetics can be applied toward the epidemiological and transmission-related goals outlined by the International Centers of Excellence in Malaria Research groups recently established by the National Institutes of Health.

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Finally, we propose genomics is a research area that can promote coordination and collaboration between various ICEMR groups, and that working together as a community can significantly advance the value of this information toward reduction of the global malaria burden.

Keywords

malaria; genomics; population genetics; *Plasmodium falciparum*; *Plasmodium vivax*; International Centers of Excellence for Malaria Research

INTRODUCTION

The publication of the *P. falciparum* genome sequence in 2002 (Gardner et al., 2002) followed by the *P. vivax* genome sequence in 2008 (Carlton et al., 2008) came with the hope and expectation that knowledge of the genomic sequences for these human malaria pathogens would both increase our understanding of the biology of these organisms and aid in development and deployment of interventions such as drugs and vaccines (Butler, 2002; Winzeler, 2008). These original genome sequences have certainly increased our understanding of parasite biology, elucidating the basic genomic structure (Carlton et al., 2008; Carlton et al., 2002; Gardner et al., 2002; Pain et al., 2008) and allowing comparisons between species (Carlton et al., 2008; Carlton et al., 2002; Kooij et al., 2005; Pain et al., 2008) to determine shared pathways among *Plasmodium* species that are unique to malaria. Functional genomic studies including investigation of transcription (Bozdech et al., 2003; Duraisingh et al., 2005; Foth et al., 2008; Freitas-Junior et al., 2005; Le Roch et al., 2003; Otto et al., 2010; Raabe et al., 2010), proteomics (Florens et al., 2002; Hall et al., 2005; Khan et al., 2005; Lasonder et al., 2002; Lasonder et al., 2008; Patra et al., 2008; Sinden, 2009; Wuchty et al., 2009), and metabolism (Besteiro et al., 2010; Olszewski et al., 2010; Olszewski et al., 2009; Teng et al., 2009) have also provided key insight to the basic biology of the parasite. However, the promise of simply gleaning this genomic information for development of effective drugs and vaccines has not been realized. Possibly the major advancement in the era of genomics applied to malaria is the realization that parasites from distinct geographical origins have different evolutionary histories shaped by their environment including the human host, the mosquito vector, and intervention strategies applied to those parasite populations (Conway, 2007; Escalante et al., 2004) including drug treatment (Anderson et al., 2011). This key observation implies that parasites can evade selective pressures either natural or applied and that patterns in genetic variation can recount this history. Thus, one can use knowledge about parasite genetic diversity to monitor parasite populations as transmission changes.

Here we discuss examples of how genomic information can be harnessed to increase our understanding of parasite biology; how population genetic approaches can be applied to discover genetic variants important for parasite survival; and, how genetic and genomic diversity relates to current challenges such as drug resistance or vaccine efficacy. Finally, we discuss how genetics and genomics of the malaria parasite provide powerful tools to understand and monitor changes in parasite epidemiology and transmission; and propose that the recently formed International Centers of Excellence in Malaria Research (ICEMR) (Rao, 2011) offer a unique opportunity to ask questions both within and between geographic settings that exhibit distinct epidemiological and transmission patterns for malaria.

DISCUSSION

Biological Insights From Parasite Genome Sequence and Population Genetics-Based Approaches

Use of Genomic Sequence to Infer Function Based On Homology—Publication of the *P. falciparum* (Gardner et al., 2002) and *P. vivax* (Carlton et al., 2008) genome sequences allowed one to use homology to describe biological pathways and infer possible functions for malaria genes. In this manner, genomic sequence data changed how research was executed such that scientists first sat down at the computer for an *in silico* experiment using knowledge from other organisms to query the parasite genome. Databases, notably PlasmoDB.org (2001; Aurrecochea et al., 2009; Bahl et al., 2002), did much to make use of homology approaches accessible to all researchers seeking biological understanding of this human pathogen. These homology searches allowed low-hanging fruit to be harvested, and identified novel biological pathways present in the parasite that might be useful as targets for chemotherapeutic interventions. For example, an enzyme involved in the biosynthesis of isoprenoids was identified in the parasite using genomic sequence information (Jomaa et al., 1999), and inhibited by the drug fosmidomycin. Subsequent investigation used genome-based approaches to identify the target of fosmidomycin resistance, the *pfdxr* gene (Dharia et al., 2009). Similarly, this catalog of genes facilitated use of comparative genomics and synteny to hypothesize functional roles for genes based upon information obtained from other organisms (Bethke et al., 2006; Carlton et al., 2005; Carlton et al., 1999; Carlton et al., 1998; Hunt et al., 2004; Kooij et al., 2005; Thompson et al., 2001). However, one key limitation of this approach is that only about half of the genes identified for *P. falciparum* have been assigned a known function based upon homology with other organisms. Thus, use of homology to discern function is limited and lack of functional prediction remains a key challenge for the use of genomics. One strategy to overcome this need to have functional predictions for a genetic locus is to use genetic variation to infer the biological importance of that genetic locus. These strategies rely on population genetic approaches that identify variants under selection, thus of likely biological importance (Jeffares et al., 2007; Mu et al., 2007; Sabeti et al., 2002; Volkman et al., 2007).

Population Genetic Approaches to Malaria—The genome sequence of *P. falciparum* allowed development of genotyping strategies to describe patterns of genetic variation in the parasite (Jeffares et al., 2007; Mu et al., 2007; Volkman et al., 2007). Information about *P. vivax* genome structure is quickly following suit (Carlton et al., 2008; Dharia et al., 2010; Westenberger et al., 2010), but much of the work described in this review will focus on *P. falciparum*. These genotyping tools include a number of array-based methods to query variation across the genome and assess from hundreds to thousands of markers depending upon the array (Dharia et al., 2009; Jiang et al., 2008; Kidgell et al., 2006; Mu et al., 2007; Neafsey et al., 2008; Tan et al., 2009; Van Tyne et al., 2011). These arrays also capture different information about genetic variants depending upon the specific technology, including single nucleotide polymorphisms (SNPs), microsatellite variants (MSVs), insertions or deletions (indels), and copy number variants (CNVs). Although whole genome array methods remain valuable, recent scientific and informatics advancements make sequencing the parasite genome both technologically and financially feasible. Genotyping or sequencing information from populations of parasites now allows determination of parasite population structure and linkage disequilibrium (Jeffares et al., 2007; Mu et al., 2007; Volkman et al., 2007), identification of signatures of selection (Ferdig et al., 2004; Ferdig and Su, 2000; Jiang et al., 2011; Li et al., 2011; Neafsey et al., 2008; Reilly Ayala et al., 2010; Samarakoon et al., 2011; Su et al., 1999; Su et al., 2007; Van Tyne et al., 2011; Yuan et al., 2011), and if phenotypic information is available, execution of genome-wide association studies (Mu et al., 2010; Nair et al., 2003; Van Tyne et al., 2011; Wootton et al.,

2002; Yuan et al., 2011) or linkage studies (Ferdig et al., 2004; Ferdig and Su, 2000; Jiang et al., 2011; Li et al., 2011; Reilly Ayala et al., 2010; Samarakoon et al., 2011; Su et al., 1999; Wootton et al., 2002; Yuan et al., 2011). However, before we consider these approaches and what they have revealed about parasite biology, we discuss two key challenges or limitations for genomic studies involving samples and phenotypes.

Challenges for Population Genetic Approaches for Malaria—One of the major limitations of malaria parasite genomic studies to date is the paucity of appropriate samples and their connection to clinically relevant phenotypes. Generally, two types of parasite samples are used for genomic studies—culture adapted *P. falciparum*, or direct patient samples either from *P. falciparum* or *P. vivax*, with each of these sample types having caveats. Culture adapted parasites are both technically challenging and require significant resources. However, once adapted these parasites can be easily assayed for *in vitro* phenotypes and provide ample DNA devoid of human material that can be easily sequenced. Although methods for *P. falciparum* culturing have been available for some time (Trager and Jensen, 1976), culture systems for *P. vivax* have proved more challenging. Renewed efforts to develop such a culture system leverage stem cells to produce the required reticulocytes for supporting *P. vivax* growth in culture (Bei et al., 2010; Giarratana et al., 2005; Panichakul et al., 2007; Tamez et al., 2009). An additional concern is the consequence of the culture adaptation process on the selection of either specific parasite types that survive (Nsobya et al., 2008), or possibly the alteration of the genomic composition of the parasite during the adaptation process (Pologe and Ravetch, 1988). Although this remains an important question, genomic tools are now available to compare direct patient isolates with culture-adapted parasites from the same infected host to determine and assess the impact of any alterations occurring during the culture adaptation process.

Direct patient samples may be easier to obtain, but often come as a mixture of parasite types, are available in limited quantities, are typically contaminated with human DNA, and they are usually either not connected to a phenotype, or connected to a phenotype that has only been tested once. Although a number of challenges remain, such as how to evaluate single parasite genomes from mixtures of parasites often found in direct patient samples, population studies are now feasible. Depletion of human material from the blood sample is possible, but one technological advance that has opened the door for using direct patient material is hybrid selection (Gnirke et al., 2009; Melnikov et al., 2011) whereby DNA samples can be enriched for malaria DNA over human material after collection. Use of direct patient samples will be critical for advancing our understanding of malaria in the natural setting.

The second challenge is that of phenotype. A number of defined *in vitro* phenotypes, such as drug resistance, invasion, gametocyte production, or metabolism, can be robustly and reliably assessed among culture-adapted parasites. However, defining and quantifying phenotypes from patients is significantly more challenging. Two obstacles associated with defining clinical phenotypes include the confounding effect of the host, and the inability to re-test phenotypes, thus likely these studies will require additional sampling to increase power. Some population genetic strategies do not require phenotypic information to identify biologically important variants (Sabeti et al., 2002) and may be useful when phenotypes are lacking. Despite these obstacles, genomic-based studies have identified new variants associated with important phenotypes like drug resistance (Mu et al., 2010; Van Tyne et al., 2011), and ICEMR groups can participate in these studies by obtaining direct patient samples with corresponding clinical phenotypes.

Population Genetic Approaches Applied to Malaria—Population genetics-based approaches have helped discern population structure including allele frequencies within and

between populations and the degree to which neighboring alleles are correlated (linkage disequilibrium, or LD) (Jeffares et al., 2007; Mu et al., 2007; Mu et al., 2010; Neafsey et al., 2008; Van Tyne et al., 2011; Volkman et al., 2007). With the few hundred genomes evaluated for population structure and LD we can discern that large-scale structure follows continents (Mu et al., 2005; Neafsey et al., 2008), with population differences in Africa being very small to undetectable, while in Asia, local population structure occurs even within a given country. LD is consistent with these patterns in that there is very little LD in Africa (Anderson et al., 2000), while it is somewhat higher in Asia (Pumpaibool et al., 2009) and even more so in the Americas (Machado et al., 2004). Population structure and LD are important because they inform how many markers are needed to see differences between populations and are useful when applied to identify signatures of selection.

Selective pressure on the parasite genome from environmental influences such as the host immune response or anti-malarial drug use, results in distinct patterns of parasite genetic variation. For example, host immunity selects variants under balancing selection (Conway, 2007), evident by high levels of polymorphism. Studies have shown that an unusually large number of genes are under balancing selection in the *P. falciparum* genome (Van Tyne et al., 2011), suggesting that the human immune system is a powerful force that shapes the genetic variation observed in the extant parasite population. Alternatively, directional selection creates a selective sweep (Smith and Haigh, 1974), whereby advantageous variants are found in high prevalence in the population that exhibits the benefit from those variants. Genome-wide scans for selective sweeps indicate that drugs are the key driver for directional selection, evidenced by the *pfcr* (Wootton et al., 2002) and *dhfr* (Nair et al., 2003) loci. Genome wide comparisons of diversity (within population, measured by π) and divergence (between populations, measured by F_{ST}) identify genetic loci that are more likely under this diversifying selection. From these analyses, known vaccine candidates are identified, as well as a number of novel loci (Van Tyne et al., 2011) recognized by the human immune system in a person infected with malaria (Mu et al., 2007).

Genome-dependent methods involving Comparative Genome Hybridization (CGH) (Dharia et al., 2009; Jiang et al., 2008; Kidgell et al., 2006; Tan et al., 2009); Genome wide Association Studies (GWAS) (Mu et al., 2010; Van Tyne et al., 2011; Yuan et al., 2011); and, linkage studies using quantitative trait loci (QTL) mapping (Ferdig et al., 2004; Su et al., 2007) have revealed new candidate loci involved in drug resistance and identified targets of new anti-malarial agents. For example, CGH approaches identified a novel CNV in the *P. falciparum* genome corresponding to the first gene in the folate pathway, GTP cyclohydrolase (*gch1*) (Kidgell et al., 2006) and this CNV was later validated (Nair et al., 2008) as being associated with antifolate use in *P. falciparum* in Thailand. Similarly, whole-genome genotyping approaches have been used to successfully identify the causal mutation associated with drug resistance when parasites have been selected for resistance to a number of anti-malarial agents (Dharia et al., 2009; McNamara and Winzeler, 2011; Nam et al., 2011; Rottmann et al., 2010; Wu et al., 2011; Yeung et al., 2010).

GWAS are very valuable for identifying genetic variants associated with key phenotypes, especially when about half the genes in *P. falciparum* have no functional annotation. One of the first association studies in malaria used microsatellite markers to find genomic regions connected to chloroquine resistance and identified a large selective sweep centered on *pfcr* (Wootton et al., 2002). Recently, several groups have carried out GWAS searching for novel variants associated with drug resistance using whole genome arrays (Mu et al., 2010; Van Tyne et al., 2011; Yuan et al., 2011). Generally these studies produce long lists of candidate loci hypothesized to be involved in the phenotype of interest; and, a major challenge is how to evaluate these candidate loci and determine which ones to pursue with functional follow up. One study to date has identified a candidate drug resistance locus using a GWAS; then,

determined the gene product (PF10_0355) is indeed associated with specific anti-malarial treatments using a transfection approach (Van Tyne et al., 2011).

Linkage analysis using a series of genetic crosses has identified biological traits related to drug response (Ferdig et al., 2004; Hayton and Su, 2008; Nkrumah et al., 2009; Sanchez et al., 2011; Wellem's et al., 1991), pathogenesis (Hayton et al., 2008), or infectivity in mosquitoes (de Koning-Ward et al., 2008; Furuya et al., 2005; Vaidya et al., 1995). Genomic markers originally provided through microsatellite variants (Su et al., 1999) and now provided through array and sequence data allows one to localize distinct parental traits among progeny parasites. For example, linkage studies identified the *pfhr-1* gene encoding a putative Na(+)/H(+) exchanger associated with quinine resistance (Bennett et al., 2007; Ferdig et al., 2004). Subsequent studies found that *pfhr-1* along with other loci may modulate quinine resistance using transfection approaches (Nkrumah et al., 2009), and that at least in some studies (Briolant et al., 2011; Pelleau et al., 2011; Sinou et al., 2011) *pfhr-1* variants track with quinine resistance in *P. falciparum*.

Genomic analysis now allows discovery of candidate loci either under selection or associated or linked to important phenotypic traits; but, functional follow up and validation of candidate variants is paramount to demonstrating the value of these genomic studies to find biologically interesting and relevant loci. Although potentially powerful approaches, both variant discovery and functional follow up using transfection approaches is not currently feasible in field-based settings. However, as new variants associated with key clinical phenotypes are identified, field groups including ICEMRs can validate these loci in independent parasite populations in the natural setting using field-deployable genotyping assays to determine whether candidate variants modulate the trait under investigation. Over the next several years, genome-based approaches are likely to advance our understanding of mechanisms of drug resistance as well as inform strategies to curtail that drug resistance.

Application of Genomics to Transcriptomics, Proteomics, and Metabolomics

—Genomic information has elucidated both transcriptional and epigenetic mechanisms of regulating gene expression using DNA microarrays (Bozdech et al., 2003; Le Roch et al., 2003) and RNA-Seq approaches (Otto et al., 2010). These studies reveal a highly ordered cascade of gene expression across the parasite lifecycle (Bozdech et al., 2003; Le Roch et al., 2003); that a major class of molecules regulating gene transcription are the AP2 (Apicomplexan Apetala2) proteins (Llinas et al., 2008); and that epigenetic regulation involves methylation that influences chromatin remodeling and var gene expression (Duraishigh et al., 2005; Freitas-Junior et al., 2005). Recently, DNA microarrays identified a family of long non-coding RNA (lncRNA) molecules (Broadbent et al., 2011) proposed to play a key part of modulating parasite virulence by contributing to a mechanism of antigenic variation that allows parasites to evade host responses (Bright and Winzeler, 2011). Transcriptional patterns have been interrogated directly from patients to help understand how parasites respond to host immunity, for example (Daily et al., 2007; Lemieux et al., 2009; Mackinnon et al., 2009). Toward illumination of patterns of immunity, protein microarrays based upon the genomic sequence are now being used to analyze host immune responses (Crompton et al., 2010), which may help in understanding how the human immune system responds to infection and modulates disease. Similarly, genomics has helped identify novel pathways of parasite metabolism including the clever use of the carbon cycle that may be key to its survival in response to environmental conditions (Besteiro et al., 2010; Olszewski et al., 2010; Olszewski et al., 2009; Teng et al., 2009). As immune correlates of transmission or disease are identified, ICEMR teams should be poised to test their usefulness to advance our understanding of human immunity, monitor vaccine success, or diagnose malaria. These concepts are discussed in the next section where we provide a framework for using genomic information to understand and monitor changes in malaria

epidemiology and transmission, and discuss opportunities for the ICEMR projects toward these efforts.

Application of Population Genetics Toward Understanding and Monitoring Changes in Malaria Epidemiology and Transmission

Information about parasite genetic diversity can be used to advance our understanding of parasite biology and discovery candidate loci associated with important biological processes such as drug resistance. However, application of population genetic approaches to questions of malaria epidemiology and transmission is perhaps most promising. In this section we outline three project areas for ICEMR groups involving application of genetic or genomic strategies toward reduction of malaria transmission. These include: use of known molecular biomarkers to assess host, vector, or parasite genotypes; use of genomic approaches to identify new variants associated with anti-malarial drug resistance; and, use of genomic, immunologic, or gametocyte-based approaches as correlates of transmission intensity to assess how interventions impact malaria epidemiology and transmission in the various ICEMR sites.

Use of Known Molecular Biomarkers to Assess Host, Vector, or Parasite Genotypes

A number of molecular biomarkers have been identified in the human host, the anopheles vector and in the *P. falciparum* parasite that are linked to the likelihood of infection or drug resistance. Assays for these molecular biomarkers are now available and one immediate opportunity for ICEMR groups as well as other field-based research groups is to utilize these genotyping assays to improve malaria control efforts. For the human host a number of key variants in hemoglobin are associated with protection from malaria infection, including variants in glucose-6-phosphate dehydrogenase (G6PD) (Hedrick, 2011; Tishkoff et al., 2001). Certain anti-malarial drugs such as primaquine, which provides radical cure of *P. vivax* infection, cannot be used in patients with G6PD deficiency, as they have increased susceptibility to hemolysis (Burgoine et al., 2010). For the anopheles vector, key molecular variants for genotyping include molecular subtyping of vector populations (Bass et al., 2007b; Henry-Hallidin et al., 2011) and identification of variants associated with insecticide resistance (Bass et al., 2007a; Hemingway et al., 2004; Kazanidou et al., 2009). For the parasite, a number of variants associated with drug resistance have been identified including SNPs in *pfert* (Djimde et al., 2001), *dhfr* (Cowman AF, 1988; Peterson et al., 1988) and *dhps* (Triglia et al., 1997); SNPs or CNVs associated with *pfmdr1* (Foote SJ, 1989; Wilson et al., 1989; Wilson et al., 1993); and, CNVs associated with *gch1* (Kidgell et al., 2006; Nair et al., 2008). Furthermore, parasite speciation genotyping—for example, to identify *P. vivax* among infections in populations where it was previously thought not to cause infection (Menard et al., 2010) and identify contributions of different parasite species in mixed infections—can now be accomplished through molecular genotyping (McNamara et al., 2006; Rosanas-Urgell et al., 2010; Snounou et al., 1993a). Knowing human G6PD variants could extend use of primaquine for treatment of *P. vivax*; and, monitoring frequencies of insecticide or drug resistant variants could guide best practices for use of malaria control measures such as indoor residual spraying or intermittent preventative treatment of malaria in pregnant women.

The genomic era has increased technology developments and created a number of new field-deployable genotyping tools to rapidly and inexpensively genotype these and other variants for field-based research and surveillance. Field deployable assays are available for both vector and parasite speciation, as well as insecticide and drug resistance markers, and include but are not limited to High Resolution Melting (HRM) (Andriantsoanirina et al., 2009; Daniels R, 2008) and Luminex (McNamara et al., 2006) methods now used by several ICEMR groups. These assays can assess current levels of insecticide and drug resistance

alleles; track the spread of such alleles to better inform which chemotherapeutic agents are most effective; and inform policy for their use. For example, studies have shown changes in allele frequencies for *pfcr* or *dhfr* in response to diminished use of chloroquine (Laufer et al., 2006) or increased use of pyrimethamine (Harrington et al., 2009), respectively, suggesting that cycling of anti-malarial drug use in an informed manner may extend the usefulness of our limited arsenal of anti-malarial agents. Knowledge of insecticide or drug resistance marker prevalence is critical for assessing the effectiveness of intervention strategies now being applied in field settings, and their impact on selecting insecticide or drug resistant variants that could undermine control efforts.

Use of Genomic Approaches to Identify New Variants Associated with Anti-Malarial Drug Resistance—Increased genomic and population genetic knowledge for both the anopheles vector and malaria parasites can be used to identify novel variants associated insecticide or drug resistance. For *P. falciparum* it has been reported that artemisinin resistance is emerging in Southeast Asia based upon observations of delayed parasite clearance rates in some infected individuals (Anderson et al., 2010a; Anderson et al., 2010b; Dondorp et al., 2009; Noedl et al., 2008). For *P. vivax* we do not know the mechanism of chloroquine resistance (Baird et al., 1991), nor have biomarkers for this trait. Finally, longer-acting partner drugs that are combined with shorter-acting artemisinin-based compounds in ACTs are vulnerable to resistance since they are present as monotherapy once the artemisinin compound clears. Yet, we know little about the precise mechanisms for many of these partner drugs such as amodiaquine and lumefantrine. Population genetic knowledge and tools now afford an opportunity for ICEMR groups to both identify parasites associated with these clinical phenotypes, and help with the larger effort to identify the corresponding genetic variants.

History shows us that use of anti-malarial drugs selects variants that confer drug resistance. Population genetic approaches can detect these variants, with ICEMR groups poised to collect useful information about changing phenotypes along with samples to genotype. For example, currently there is no information about parasite clearance rates with ACT use in Africa. And although there is no evidence of delayed parasite clearance in African populations, baseline data is important for detecting any future change should artemisinin resistance emerge in Africa. ICEMR groups can work together to apply common protocols for measuring parasite clearance rates and collect corresponding samples for larger community efforts attempting to identify genetic variants associated with artemisinin resistance.

Although it is unclear whether delayed parasite clearance rates ascribed to artemisinin resistance will be represented as a change in IC₅₀ values for artemisinin-based compounds, to date there is no evidence that extended clearance rates correspond to changes in parasite drug responses *in vitro*. Nevertheless, monitoring parasite responsiveness to both the artemisinin-based compounds as well as partner drugs used in ACTs may be important for identifying any future diminished drug responsiveness. While accurate determination of IC₅₀ values for parasites taken directly from patients may be challenging, a number of field labs perform these assessments routinely. These assays can provide an early warning system to detect parasite drug resistance before reduced clinical effectiveness is evident, since drugs are tested individually in culture, while used in combination clinically. ICEMR groups now have the opportunity to monitor parasite drug resistance *in vitro*, and simultaneously evaluate genetic changes associated with diminished partner drug responsiveness. Although some genetic variants, such as within the *pfmdr1* locus, have been associated with partner drugs such as amodiaquine or lumefantrine, these mechanisms of drug resistance are not fully understood. Furthermore, we lack specific markers for easy tracking of parasite

responsiveness to partner drugs in the field. Thus, finding variants associated with partner drug resistance is a promising research opportunity for ICEMR groups.

Use of Known Molecular Biomarkers to Monitor Changes in Parasite Population Structure that Reflect Malaria Transmission—

A large number of factors contribute to transmission, which underpins malaria infection. A number of genomic, immunologic, and gametocyte-specific parameters, currently able to be measured in the field, provide possible correlates of malaria transmission. Parasite population structure reflects the number of different parasite types infecting humans in a given population, and thus reflects the relative level of malaria transmission intensity. Two specific population genetic parameters expected to change as transmission declines include complexity of infection (COI) and frequency of clonal infections. COI represents the number of different parasite genomes within an infected individual. As the number of parasite types diminish in a population with declining transmission, COI will correspondingly decrease toward one. As COI gets very low there is less opportunity for recombination between parasite types and parasites will look more genetically homogeneous, coincident with a rise in the number of clonal parasite types observed when transmission is severely reduced. Currently COI can be measured in the field by genotyping highly polymorphic loci including *msp1*, *msp2*, and *glurp* (Snounou et al., 1993b), but advancements in sequencing and informatics is moving this analysis to a population-based level albeit using methods presently only available at larger sequencing centers. One technology currently deployed in the field is the molecular barcode, which queries a set of independent SNPs and uses this information to create a signature for a parasite type (Daniels R, 2008). When transmission is relatively low, the molecular barcode tool can follow specific parasite types in the field setting to assess the effectiveness of drugs or vaccines. Similarly, it can identify whether new malaria cases are due to local rebound or importation when transmission is very low, information that impacts malaria control efforts. These genotyping tools are now deployed in the field and can assess the consequences of interventions on parasite population structure as a possible surrogate for malaria transmission.

In addition, information about immunological and gametocyte-specific parameters can be obtained in field-based centers and one key challenge is connecting all these measures with independent assessment of transmission such as the entomological inoculation rates. For example, specific antibody signatures have been used to examine malaria transmission (Cook et al., 2011; Drakeley and Cook, 2009) and identify possible hotspots of epidemic transmission (Bejon et al., 2011; Bousema et al., 2010) in some settings. Opportunities for ICEMR and other field groups include investigation of immune responses under different malaria transmission settings and testing the correlation between immune indicators and malaria transmission patterns (Barry et al., 2011; Doolan et al., 2008; Tan et al., 2011). Expression patterns of gametocytes and evaluation of gametocyte carriage related to transmission is another opportunity for field-based research groups (Adjalley et al., 2011; Bousema and Drakeley, 2011; Karl et al., 2011; Liu et al., 2011). Ideally these tools can quickly and reliably assess current levels of malaria transmission and monitor changes as interventions are applied or as environmental conditions (i.e. changing rainfall) impact these dynamics. ICEMR groups have the opportunity to assess different tools to monitoring malaria transmission, including those that detect changes in population structure, immunological indicators, and gametocyte carriage rates, across the various malaria-endemic settings with specific epidemiological parameters. ICEMR groups can then test whether these assays are useful for monitoring malaria epidemiology and transmission in their various settings. Knowledge of current transmission dynamics as well how environmental factors including changes in rainfall or the application of various intervention strategies impact these transmission dynamics will be paramount to successful reduction of malaria disease burden worldwide.

CONCLUSION

In the past several years the genome sequence for the human malaria parasites, *P. falciparum* and *P. vivax*, have been discerned. However, this information has not readily been leveraged to reduce the global burden of malaria. Despite this, genomic information has significantly advanced our understanding of parasite biology and knowledge about parasite genetic diversity is now being used to aid discovery of vulnerable targets for intervention strategies, such as drugs, vaccines or insecticides, and to identify biomarkers for diagnosis, surveillance and monitoring of malaria infections in humans or mosquitoes. The formation of the ICEMR network spanning many malaria-endemic regions offers new opportunities for apply these evolving genomic approaches to enhance our understanding of malaria epidemiology and transmission. This understanding is paramount to any successful effort to reduce malaria disease burden as we work toward elimination and ultimately eradication of malaria.

Although the ICEMR structure offers a new opportunity for researchers and scientists to work together across regional centers each characterized by distinct epidemiological and transmission patterns, success of this network has a number of challenges. Each group thus far has been working independently to get their site and field teams working together. This is important work, but we suggest it is equally important to leverage the value of a network of investigators and work together on common research projects that benefit from a larger collective effort. Transparent discussions about governance regarding ICEMR network studies such as how to share samples and data will be required; and, models such as consortiums or multi-center trial groups will likely be helpful in facilitating these processes (2008). Partnerships will be key to the success of this network, both among different ICEMR groups and between northern and regional partners. For example, discovery of novel variants related to drug resistance currently requires collaboration between northern (i.e. outside malaria endemic areas) and regional (i.e. inside malaria endemic areas) partners. Regional partners can best identify the relevant phenotypes and the corresponding samples; northern partners can utilize this information to identify novel candidate variants associated with these relevant phenotypes; then, regional partners can validate these variants in the field, and assess their value for monitoring and surveillance purposes. Key to success of these partnerships is training. For example, ultimately sequence data will be freely available through community projects supported by large funding agencies. One critical measure of success of ICEMR groups will be training regional partners how to use genotyping and sequencing information for the discovery of novel candidate variants on their own. Partnerships will be critical for successful transfer of skills to sustain and extend these research efforts within malaria endemic areas.

In this review we have proposed three research areas that could be carried out across the different ICEMR groups. Two of these efforts can be implemented immediately, namely surveillance of key molecular variants in the human, mosquito and parasite populations such as those related to insecticide and drug resistance. The other is using indicators of changing transmission—genomic, immunological, and gametocyte-specific—connecting these parameters with independent measures of transmission intensity as interventions are applied. The third proposal, to work together to identify novel variants associated with drug resistance (artemisinin and partner drug resistance for *P. falciparum* and chloroquine resistance in *P. vivax*) has a longer timeline, but now is the time to gather baseline data to be able to identify these novel variants over the next 5 to 10 years. Putting in the effort to systematically collect and process genetic and genomic information now will enable us to better understand malaria epidemiology and transmission. Furthermore, application of genomics to field investigations by the ICEMR groups can assess the impact of changing environmental conditions either natural (e.g. rainfall, immunity) or applied (e.g. drugs,

vaccines) to identify successful and sustainable approaches most likely to reduce the global malaria burden as we work toward elimination and eradication of these important human pathogens in resource-limited environments.

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