THE NEED OF GENOME-WIDE ASSOCIATION STUDIES FOR RESISTANCE TO MALARIA AND SUSCEPTIBILITY TO OTOTOXICITY OF ANTI-MALARIAL DRUGS IN ETHIOPIA

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Abstract

Malaria is a major cause of morbidity and mortality in Africa including Ethiopia. In addition to environmental factors, genetics have important contribution to determine variations among individuals for resistance to malaria infection, severity of the disease and susceptibility to adverse drug reactions. Although candidate gene studies partly uncover genetic variations responsible for resistance to malaria infection, limitations such as lack of reproducibility, discordant reports, and dependence on a priori knowledge of the disease, have been recognized. This calls for genome-wide association study (GWAS), which is comprehensive and unbiased approach to understand the complex nature of the disease. GWAS gives an opportunity to explore common variations on the entire genome for association with the trait. This approach could elucidate novel genes and biological pathways for clinical applications on further studies. The successes of GWAS approach to identify responsible genetic variants have been illustrated by various studies on common diseases, drug responses and serious adverse reactions.

To this effect, undertaking GWAS on populations with massive burden of malaria such as Ethiopia, on genetic risk variants for infection and serious adverse reactions to anti-malarial drugs is important. Identifying variants for resistance to infection may provide molecular basis of protective immunity for prospective vaccine development.

One of the reported adverse effects of anti-malarial drugs, particularly quinine is ototoxicity. Although this adverse effect generally considered reversible, there are reports on marked hearing impairment or permanent hearing loss that varies among patients treated with the drugs. Exploring genetic variants responsible for ototoxicity through GWAS could help to screen high risk patients for alternative treatment.

Keywords: malaria, genome wide association study (GWAS), ototoxicity, Ethiopia
Introduction
Malaria is a major public-health problem and the most important tropical disease responsible for significant health related and social burdens [1]. It is a life-threatening disease caused by parasites of the genus Plasmodium (P. falciparum, P. vivax, P. ovale and P. malariae), which invade and asexually reproduce in human erythrocytes. The parasite is transmitted from an infected person to another by blood-sucking Anopheles mosquitoes. Plasmodium falciparum malaria is the most serious form of malaria which is the major cause of morbidity and mortality, particularly in endemic areas of sub-Saharan Africa [2]. In spite of recent evidence of successful control in some countries, P. falciparum malaria still constitutes a major cause of child morbidity and mortality [3]. Annually, it kills about a million children and causes debilitating illness in more than half a billion people in the world [4].

Malaria is a disease with many genetic and environmental determinants influencing the observed variation in response to infection, disease progression and severity.

Malaria is the leading communicable disease in Ethiopia, the Eastern Horn of Africa [5]. About 75% of the geographic area of the country has significant malaria transmission risk, with about 68% of the total population living in these risk areas [5]. According to the Federal Ministry of Health [6], malaria is the major cause of outpatient visits, accounting for 14% of all visits with 9% admissions, and it is one of the top ten causes of inpatient deaths among children less than five years of age. The FMOH estimates that there are about 5–10 million clinical malaria cases each year [6]. Malaria Indicator Survey done recently showed that 4.5% of children were positive for malaria using rapid diagnostic test, and P. falciparum constituted 77% of these infections [7].

Currently, artemisinin derivatives are the first line anti-malarial drugs used for the treatment of P. falciparum malaria in Ethiopia [8]. Quinine is the preferred alternative drug of choice when artemisinin derivatives are not available or contraindicated.

**Genome wide association study as a tool to study common diseases**
Approaches to map genes that underlie common diseases and complex traits fall broadly into two categories: candidate-gene and genome-wide studies [9]. Genetic contributions to infectious diseases such as malaria in human populations are supported by epidemiological evidences [10]. The majority of genetic epidemiology studies, including studies for susceptibility to severe malaria infection, have used candidate gene approach which is restricted to specific genes based on their presumed functional relevance to a disease. Although there are successful contributions of candidate gene studies in uncovering genetic risk factors to diseases, some of the results of candidate gene studies failed to be replicated [11] and others led to discordant results [12, 13]. In addition, the discovered genetic factors account only for a small proportion of the variations implicating that many genetic variants remain to be uncovered [10, 14]. In this sense, approaches such as genome-wide association studies could have a great potential to understand the complexity of common diseases [11].

Genome-wide association studies (GWAS) are genetic epidemiological studies in which a dense array of genetic markers which capture a substantial proportion of common variations in genome sequence are typed in a set of DNA samples that are informative for a trait of interest [15]. The ultimate goal of GWAS analysis is to uncover DNA sequence variants that affect an individual’s risk to a disease or serious adverse effect of drugs through the detection of association between genotype frequencies and trait status. GWAS explores the entire human genome for causal genetic variants of the observable trait [11]. Because no assumptions are made about the genomic location of the causal variants, this approach could exploit the strengths of association studies without having to guess the identity of the causal genes [9]. The GWAS approach therefore represents an unbiased scan of the genome yet fairly comprehensive option that can be attempted even in the absence of evidence regarding the function or location of the causal genes. In contrast to a candidate gene approach which is limited to the current knowledge of pathogenesis of diseases, GWAS approach allows the identification of novel susceptibility variants that promise to provide better biological understanding of phenotypes [16]. GWAS enables to elucidate the involvement of multiple genes or previously unsuspected biological pathways in disease development [17].

GWAS is an increasingly popular approach for identifying genetic factors influencing common complex traits [18]. The popularity of this approach reflects the major advances in the technology for high-throughput genetic analysis which is now associated with very low error rates. High-throughput genotyping platforms have driven down the costs of genotyping studies on a genome-wide scale [18, 19]. Recent successes in the identification of susceptibility variants that underlie many
important biomedical phenotypes through GWAS have increased confidence that this information can be translated into novel biological insights and clinically beneficial improvements in disease management [15, 17].

GWAS make use of abundant and easily genotyped molecular markers, single nucleotide polymorphisms (SNPs), which are single-base substitutions with minor allele frequencies above one percent [11, 20]. Nowadays, data generated after sequencing of human genome and the HapMap project had identified millions of SNPs facilitating the implementation of GWAS. The fact that there is a high degree of correlation between adjacent SNP variants allows the majority of common variations to be assayed with half a million or more correctly chosen proxy SNPs [9, 20].

In GWAS, once biological samples from cases and controls of the phenotype under consideration are collected, the DNA will be extracted and genotyping will be performed on each sample on SNP microarray [21]. Then, for each genetic marker that passes the rigorous quality control measures, a statistical test will be performed to determine whether the alleles or genotype frequencies at the marker can predict the phenotype. If the test reaches statistical significance after accounting for multiple testing, then the variant is deemed to be associated with the phenotype. To reduce the false positive rate and increase power, the study could be done in two or more independent data sets [9].

If a SNP in a genomic region is significantly associated with the phenotype, additional markers can be genotyped in that region, termed fine-mapping, to identify the responsible gene and variant to arrive at biological insights [21].

The analysis of GWAS has already enabled the successful identification of novel genes for complex disorders such as Crohn’s disease, rheumatoid arthritis and diabetes [22], for susceptibility and disease progression of infectious diseases such as HIV [23], hepatitis-B [24], tuberculosis [25] and for responses to drug treatment [26-28] among others. The successful applications to identify novel susceptibility genes for complex polygenic diseases and for drug responses resulted in an interest in applying GWAS to uncover genetic variants for serious adverse drug reactions [29].

The person-to-person variability of a drug response is one of the major problems in clinical practice, and could lead to therapeutic failure or adverse effects of drugs in individuals or subpopulations of patients (30). Potential risk factors for drug toxicity include age, gender, nutritional status, co-morbidities, drug interactions, and lifestyle variables such as alcohol consumption [31]. Of even greater importance in the determination of individual risk are genetic factors that affect the kinetics and dynamics of drugs [32]. In general, genetic factors are estimated to account for 15–30% of inter-individual differences in drug metabolism and response, but for certain drugs, this can be as high as 95% [33]. Thus, GWAS that focus on drugs that cause severe adverse effects may allow the development of diagnostic tools offering better prediction of the adverse effects.

GWAS on adverse drug reactions have now been done which include those for drug-induced myotoxicity [34], hepatotoxicity [35-37] and skin reactions [38, 39] among others. Relatively a few GWAS in pharmacogenomics have been done compared with the large numbers of disease risk GWAS [40]. However, from those published, it is evident that the potential for gaining insight into the genetic aspects of adverse drug reactions, and hence for developing tools to minimize adverse drug reactions, is very large. Thus, GWAS through their capacities to scan the majority of human genome could offer the potential to overcome the lack of reliable data on the genetics of serious adverse drug reactions [41].

GWAS for resistance to malaria infection
In malarial infection, although the virulence of the parasite, host age, state of acquired immunity and overall health status may influence the risk of disease complication [42], it has been estimated that 25% of this risk can be accounted for by variations in host genome [14]. Variations in severity of malaria infection considered as different phenotypes and have important consequences in term of morbidity and mortality [4, 42, 43]. So far, gene mutations and polymorphisms of candidate loci that have been identified to confer survival advantage in malaria infection include sickle cell trait, haemoglobinopathies and Duffy-negative blood group [44, 45]. Since host genetic factors contribute to the variability of malaria phenotypes, identifying this variability may provide clues to molecular basis of protective immunity that would be invaluable for vaccine development [46], and thus may solve challenges of drug treatment and disease control.

GWAS done in West Africa identified two novel loci associated with severe P. falciparum malaria [47]. One of the loci is linked with a gene that encodes tight-junction protein expressed on endothelial cells which might have a role in microvascular damage caused by endothelial adherence of parasitized erythrocytes.
Different populations could harbor various factors that confer resistance to malaria [48, 49]. Studies that would be done on different populations could aid in uncovering genetic factors and in investigating interactions between genes & the environment, and therefore could provide a rich resource for identifying causal variants.

Genetic variants that confer resistance to malaria infection in East Africans including Ethiopians have not been studied. Thus conducting genome wide association discovery and replication studies in Ethiopia, where there is massive burden of the disease, is highly recommended. Understanding the genetic basis of resistance to malaria infection could provide valuable insights into molecular mechanisms of protective immunity that will aid the development of vaccines.

GWAS for ototoxicity of anti-malarial drugs

Hearing loss is the most prevalent sensory impairment in humans caused by various factors including acoustic trauma, ototoxic drugs, infections and genetic predispositions [50, 51]. Ototoxicity is defined by the toxic capacity of certain drugs or toxins to inner ear structures [52]. This effect could lead, in the most severe cases, to remarkable functional impairment of hearing capability or complete deafness due to cellular degeneration of inner ear tissues [53, 54].

Hearing impairment or deafness due to ototoxicity is estimated to be 3-5% of all deafness in developing countries [55] and have been increasingly reported from different countries [56]. Economic impact of hearing loss is high because of work-related difficulties, educational limitations, social stigma & exclusion [57]. Ototoxic drugs fall into various therapeutic classes including antimalarial drugs, with different pattern of cellular damage [51]. To date, only a few of these ototoxic drugs have been investigated for the genetic risk factors and molecular mechanism of their toxicity. Ototoxicity effect of antimalarial drugs particularly quinine, which is also used for nocturnal leg cramps [58], have long been recognized [59]. Although there are limited reports on artemisinin derivatives, a report from Mozambique indicated an irreversible hearing loss in patients exposed to artemether-lumefantrine [60]. Quinine in standard therapeutic dose has been reported to cause cinchonism, a set of adverse effects consisting nausea, vertigo, dizziness, headache, tinnitus, mild to high-tone hearing impairment and blurred vision [61]. These adverse symptoms seem to increase with increasing plasma concentration of quinine [62].

Investigations indicate that quinine can act directly on cochlear outer hair cells to affect their motility and amplification performance [63]. Although it seems established that the hearing impairment by quinine is reversible, there is a report of permanent hearing loss following quinine administration [64]. There are limited studies with large sample sizes on the ototoxic effect of antimalarial drugs in humans. A study from Dar-es-Salaam showed irreversible deafness following intravenous infusion of quinine [64]. This study also showed that ototoxicity was the cause of 20% of permanent deaf cases in the study group. In another study, out of 103 patients who were diagnosed as having ototoxicity, quinine was the commonest implicated drug (19%) with mild to profound hearing loss [65].

Although the risk for developing hearing loss from ototoxic drugs is generally related to the dose, duration, frequency, and route of drug administration [66], studies showed that there is marked individual variability in these relationships [67]. There are reports which show direct genetic predisposition on susceptibility to ototoxic drugs: for example, ototoxicity induced by amino-glycosides [68, 69] and cisplatin [54, 70]. These could lead to the hypothesis that individuals having polymorphisms in one or more genes may render them susceptible to ototoxic effect of quinine as well. Identifying genetic variations for susceptibility to drug induced ototoxicity could contribute to the development of predictive markers for prevention of ototoxicity by identifying patients at risk. This will in turn help to improve the safety of the drug therapy on decision making to offer alternative treatment for those who carry the risk allele, and eventually decrease the incidence of deafness.

Since the use of antimalarial drugs is common in Ethiopia and so far there have not been studies done to identify genetic risk factors for quinine-induced hearing impairment, conducting GWAS and replication studies could be valuable. Identifying this variability could increase the knowledge of the molecular basis of quinine-induced hearing impairment and might open new perspectives for preventive and therapeutic strategies for permanent hearing loss due to medications.

Conclusion

Malaria is a major cause of morbidity and mortality in Sub-Saharan-Africa including Ethiopia. It is known that genetic variations have important contribution to determine variations among individuals for resistance to malaria infection, severity of the disease and susceptibility to adverse drug reactions.
of anti-malarial drugs such as quinine-induced ototoxicity. Therefore, performing GWAS to identify the genetic risk variants could help to provide molecular basis of protective immunity for prospective vaccine development and to screen high risk patients for alternative drug treatment.

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