Essay

Genomics Research and Malaria Control: Great Expectations

Vincent P. Alibu, Thomas G. Egwang

The protozoan parasite *Plasmodium falciparum* causes falciparum malaria, a fatal parasitic disease in humans, and is transmitted by *Anopheles* mosquito vectors (predominantly the *Anopheles gambiae* complex and *An. funestus* in Africa). There are about 300 million malaria cases and 1–2 million deaths annually, the brunt of which are borne mostly in Africa by children under 5 years of age and by pregnant women. In many African countries, malaria poses a formidable challenge to an overburdened and underfunded public health system. The current malarial control strategies consist of chemotherapy directed against the malaria parasite and prevention of mosquito vector/human contact using insecticide-impregnated bednets and, to a lesser extent, indoor residual insecticide spraying and environmental control for reducing mosquito breeding sites. There are still no malaria vaccines in clinical practice.

The Dual Problem of Drug and Insecticide Resistance

Chemotherapy (the use of drugs to target disease) is used for both treatment and prevention. Drug resistance is increasingly becoming a problem. Some of the antimalarial drugs in current use include quinolines, artemisinins, antifolates, atovaquone/proguanil, and antibiotics. Chloroquine

Vincent P. Alibu is at the Zentrum für Moleculare Biologie at the University of Heidelberg in Germany. Thomas G. Egwang, an International Research Scholar of the Howard Hughes Medical Institute, is in the Department of Medical Parasitology at the Medical Biotechnology Laboratories in Kampala, Uganda. E-mail: egwang@imul.com

DOI: 10.1371/journal.pbio.0000039

(CQ) is a cheap and widely used aminoquinoline, but CQ-resistant parasites have become ubiquitous in endemic countries and other drugs are now used much more frequently (Ridley 2002). Fansidar, a combination of sulphadoxine and pyrimethamine (SP), is a first-line treatment in several African countries, but resistance to SP is spreading rapidly. Targeting the mosquito vector with pyrethroid-impregnated bednets, in addition to chemotherapy, is an effective method of controlling malaria transmission. However, pyrethroid resistance has been reported in *An. gambiae* s.s. in West Africa, and there is concern about its emergence in East Africa (Chandre et al. 1999). Thus, the public health problem due to malaria is exacerbated by the emergence of drug-resistant parasites and insecticide-resistant mosquitoes. The clinical application of efficacious intervention tools is therefore an urgent imperative for malarial control. This brings into sharp focus the importance of genomics research for drugs, vaccines, diagnostics, and insecticides. The unraveling of the genomes of humans, *P. falciparum*, and *An. gambiae* has ushered in a new era of hope that genomics research will result in the development of new and better tools for malaria control.

Early Pickings from the Malaria Genome

The *P. falciparum* genome of 22.8 megabases (Mbp) distributed among 14 chromosomes consists of 5,300 protein-coding genes (Gardner et al. 2002). *P. falciparum* possesses a relict plastid, the apicoplast, homologous to the chloroplasts of plants and algae. The apicoplast is essential for parasite survival and functions in the anabolic synthesis of fatty acids, isoprenoids, and heme (Seeber 2003). These essential metabolic pathways are not present in humans and are therefore ideal targets for the development of safe antimalarial drugs. Inhibitors of type II fatty acid biosynthesis (triclosan and thiolactomycin) and mevalonate-independent isoprenoid biosynthesis (fosmidomycin and FR900098) with potent antimalarial activities have been identified by computational mining of the genome data. The fact that fosmidomycin has rapidly entered into clinical trials underscores the great utility of genomics research in the control of malaria (Lell et al. 2003).

Malaria Functional Genomics

About 3,200 proteins (60%) in *P. falciparum* have no known functions (Gardner et al. 2002). The greatest challenge of malarial functional genomics (the elucidation of the functions of genes encoded by an organism's genome) is to assign functions to these proteins, thus comprehensively identifying the proteins that function at various lifecycle stages and that function together to carry out particular cellular processes, e.g., red blood cell invasion, signal transduction, growth, vesicular trafficking, etc. The application of functional genomics approaches allows the properties of many genes and proteins to be assessed in parallel on a large scale. These approaches are being used to address specific questions about the biology of *P. falciparum*. Gene profiling (determining which genes are expressed) by microarray technology allows a rapid, parallel analysis of genome-wide changes in gene expression over a variety of experimental conditions (e.g., chloroquine versus saline control), tissues, and cell types; these genes can be clustered (ordered by expression pattern) to identify those that function in the same process. One of the most promising applications of microarrays is the study of differential gene expression during the complex *P. falciparum* lifecycle, specifically the formidable and challenging task of determining which subset of the 5,300 genes is represented in the transcriptome of each stage (Bozdech et al. 2003; Le Roch et al. 2003). These approaches are beginning to yield invaluable insights about new vaccine candidates, novel drug targets, and the molecular basis of drug resistance.

Proteomics is the study of all the proteins expressed in an organism. Global protein analysis offers a unique means of determining not only protein expression, but also interacting partners, subcellular localizations, and post-translational modifications of proteins of whole proteomes. Analyses of the proteomes of parasites that have been exposed to distinct environmental stimuli (e.g., chloroquine versus saline control) or that manifest distinct phenotypes (drug resistant versus drug sensitive) might also facilitate the identification of biochemical drug targets and of the specific proteins involved in drug resistance. Comparative genomics (the comparison of genomes of related species), on the other hand, will yield invaluable insights about the biology of and the pathogenesis of disease associated with different parasites, i.e., *P. falciparum*

DOI: 10.1371/journal.pbio.0000039.g001

Figure 1. Patients at Apac Hospital
Of the patients waiting at the Out-Patient Department of Apac Hospital in Northern Uganda, the majority are mothers of children under 5 years old with malaria. (Photograph by Toshihiro Horii, Department of Molecular Protozoology, Research Institute for Microbial Diseases, University of Osaka, Osaka, Japan.)

on the one hand and *P. vivax* on the other. The biology and pathology of the two parasites are quite distinct, e.g., the preference for reticulocytes (*P. vivax*) versus mature red blood cells (*P. falciparum*), the ability to cause severe (*P. falciparum*) versus mild (*P. vivax*) disease, and the implication of amino acid substitutions in PfCRT in CQ resistance in one (*P. falciparum*) but not in the other (*P. vivax*).

The Promise of Mosquito Genomics

The 278 Mbp sequence of the nuclear genome of the PEST strain of *An. gambiae* s.s. has been published in draft form and is considerably larger than the 122 Mbp assembled sequence of the fruitfly *Drosophila melanogaster* (Holt 2002). The *An. gambiae* genome includes a treasure trove of 79 odorant receptor genes and about 200 genes that encode glutathione-S-tranferases, cytochrome P450s, and carboxylesterases. These and possibly other genes probably play a critical role in human host finding and detoxification of insecticides, respectively, and could be exploited, using gene profiling, proteomics, and comparative genomics, for the development of novel mosquito repellants or traps and insecticides. The ability to introduce foreign genes into *Anopheles* vectors is an exciting advance that might facilitate the development of transgenic mosquitoes that do not transmit malaria parasites (Moreira et al. 2002). However, the future implementation of this control strategy, if current technical hurdles can be overcome, must take into consideration concerns about the environmental impact of releasing genetically altered mosquitoes.

Capacity Building in Endemic Countries

Scientists in endemic countries must be active participants in malaria genomics research and not just conduits for field materials for Northern partners. However, the reality is that there is an increasing technological gap between endemic- and developed-country researchers in the field. This needs to be urgently addressed. The World Health Organization Special Programme for Research and Training in Tropical Diseases have initiated a series of training workshops in bioinformatics in endemic countries; the Howard Hughes Medical Institute has supported one such workshop. The training must extend to other aspects of genomics and include infrastructure development.

Great Expectations

There is considerable optimism that genomics research will result in new drugs, vaccines, diagnostics, and tools for malarial vector control. Strong linkages between genomics research and national malarial control programs will facilitate the translation of research findings into intervention tools. As it is for all new technologies, it might also be important for the communities in endemic countries to have a greater awareness and understanding of genomics research. This will enhance acceptance of the products and improve informed consent. There is therefore a unique opportunity for collaborations between social-economic scientists and genomics researchers. ■

Acknowledgments

We are grateful to Drs. Lisa Ranford-Cartwright (University of Glasgow, Glasgow, United Kingdom), Ayoade Oduola and Yeya Toure (World Health Organization, Geneva, Switzerland), and Wilfred Mbacham (University of Yaounde, Yaounde, Cameroon) for critical comments on the manuscript.

References

Bozdech Z, Llinás M, Pulliam BL, Wong ED, Zhu J, et al. (2003) The transcriptome of the intraerythrocytic developmental cycle of *Plasmodium falciparum*. PLoS Biol 1: 10.1371/journal.pbio.0000005.

Chandre F, Darrier F, Manga L, Akogbeto M, Faye O, et al. (1999) Status of pyrethroid resistance in *Anopheles gambiae sensu lato*. Bull WHO 11: 230–234.

Gardner MJ, Hall N, Fung E, White O, Berriman M, et al. (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature 419: 498–511.

Holt RA (2002) The genome sequence of the malaria mosquito *Anopheles gambiae*. Science 298: 129–149.

Lell B, Ruangweerayut R, Wiesner J, Missinou MA, Schindler A, et al. (2003) Fosmidomycin, a novel chemotherapeutic agent for malaria. Antimicrob Agents Chemother 47: 735–738.

Le Roch KG, Zhou Y, Blair PL, Grainger M, Moch JK, et al. (2003) Discovery of gene function by expression profiling of the malaria parasite life cycle. Science 31 July 2003: e-publication ahead of print.

Moreira LA, Ghosh AK, Abraham EG, Jacobs-Lorena M (2002) Genetic transformation of mosquitoes: A quest for malaria control. Int J Parasitol. 32: 1599–1605.

Ridley RG (2002) Medical need, scientific opportunity and the drive for antimalarial drugs. Nature 415: 686–693.

Seeber F (2003) Biosynthetic pathways of plastid-derived organelles as potential drug targets against parasitic apicomplexa. Curr Immun Endocr Metabol Disord 2: 99–109.