

**Genomes: Host versus Parasite Genes:
Which Genome is it Best to Look in for a Solution to Malaria?
Fourth Mim Pan-African Malaria Conference
Yaounde, Cameroon
November 16, 2005**

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[START RECORDING]

FRANCINE NTOUMI, PH.D. (LAMBARENE): And I have the honor to present officer Dominick Kwiatkowski from Oxford University. He will be defining the importance of the studies on human genome and Professor Patrick Duffy from the Walter Reed Army Institute and the Seattle Biomedical Research Institute will reassure us on how the investigation on outside genome. Next slide, please? So the factors determining the severity of the disease are complex and include human hosts for the most of them and also has a balance and acquired immunity. And some how done hypothesis in 1949, it has been shown clearly that among all the infectious diseases, malaria has been the strongest selective force on human genome. And these last decades, where [inaudible] in multiple human genes have been [inaudible] to affect susceptibility to plasmodium falciparum infections. And the most that either cyto [inaudible] trait, [inaudible] [French] and in part of point of view, there is help for [inaudible] in plasmodium falciparum genome. And the expression of proteins is just specific and it has been also important sure that some positive factors associated with factor genetics of malaria, factors such as resulting in fight to adherence. Next slide, please? So, since 2002, the complexes you have in pathogenic sequence are available. And it is a real exciting time for modernized researches. And, at

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the same time a new language has been promoted in the scientific community such as—please next—genomics, transcription, proteomics, microarrays, antibody [inaudible] [inaudible] biology. And I can understand that some of us be a little bit lost or border line of these new technologies. [Inaudible - French] Please come back. [Interpreter] [Inaudible] [Interposing] of sequences are available and since that period there is a new sense of words, possibly [inaudible] genomics, microarrays, proteomics, [inaudible] bioinformatics. And it is very clear that some of us will not be able to understand some of these words. [Inaudible] as part of discussion. In the first part, Patrick Duffy and Dominick Kwiatkowski present advances in malaria genetics and proteomics reserve and human hosts in parasites. Next slide, please? And to ensure us how important how this massive research for the identification of new drug targets, identification of new malaria that's in candidates, and for the development of new and better diagnostic tools. Next slide, please? And also with this huge amount of data generated, there will—explain this. How the different results of proofs are cooperating together? And how is all this [inaudible] are coordinated? Next slide, please? And the last issue, we will talk about malaria research in Africa. Genomics, proteomics, transcription [misspelled?] in Africa. Next, please? And how

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involved are African scientists? How involved, particularly junior African scientists' discovery of drug infection research? And the question with it: Do you think that there is now with this new technology and present data between research of wealthy in Africa and researches working else at Africa and we will also share and discuss your idea about sensors or lack of existence or lack of difference in Africa. So, now I will let Patrick Duffy present his point of view and the microphone is yours.

PATRICK DUFFY, M.D. (SEATTLE): This your laser? I don't have one. Does anybody have a laser?

FRANCINE NTOUMI, PH.D. (LAMBARENE): This one works.

PATRICK DUFFY, M.D. (SEATTLE): Okay. Well, thank—no laser—thank you Francine, for that introduction and also my appreciation to the organizers of this great conference, for inviting me to participate in this session, particularly to Lars who made the invitation. It is a good opportunity to highlight some of these new tools and how they can be applied to study the parasite in the host genome, to try and find solutions and both Dominick and I agree that would sound a bit like a [inaudible] to try to say that one or the other is really not helpful in the search for solutions. But what I'm going to be talking about is using the parasite genome to find the solution and the talk is based on a couple of assumptions.

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The first one is that the genome is helpful for many things, as Francine pointed out, but I'm specifically going to be focusing on the use of the genome for a vaccine because barring the ratification of the parasite or the mosquito, the best long-term solution is going to be vaccines. And using the genome means—it means several things actually. One that I think that Dominick will be focusing on will be looking at genomic DNA to find clues to what maybe contributing to disease or immunity and another thing that using the genome means is to do special studies, either at the level of gene expression, or protein expression. And that is a lot of what I will focus on, with the parasite and then both of us, as Francine also pointed out, require quite a bit in the form of viral and dramatic tools and store and manage and analyze all the data that we're getting from these studies. And finally, for my talk, I want to holler at the fact that the genome is quite an unreality monster unless you have a framework for trying to approach the use of it. And so what I want to do is to talk about how the most efficient use of the genome is best accomplished with a model of immunity in order to do the studies. Can I have the next slide? So, when I think about free solutions that are vaccine based, the ones that primarily come to mind, are to prevent severe malaria in children, to prevent pregnancy malaria and finally the wholly goal is to prevent infection completely and

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these are the solutions that I think the parasite genome is really on its way to helping us achieve. Next slide? And the two examples that I'll talk about to kind of give concrete examples of how these tools are used is first, the effort to develop a pregnancy malaria vaccine, which involves several laboratories as does the effort to develop an attenuated parasite vaccine and I'll go through them in order. Next slide? So, this slide may remind you of a nice slide that Unger Bardone [misspelled?] showed on his talk on the first day. On the upper left hand corner you can see that there's a section of a placental villus and what we've done and what my colleague Freda's done, my colleague, has collected parasites from infected placenta and allowed them to settle on a section of infected villus and then washed away, which doesn't find any. And, as you can see, the parasites that come from the infected villus by drain along the surface, the lining of the placenta villi. And this binding is completely aggregated with the use of Chondroitinase AC. So, this is the model of immunity that I'm talking about, that's helping to guide us in the use of these tools. First of all, we've learned that there's a distinct parasite form that infects the human placenta and causes the bad outcomes of pregnancy, and that parasite form typically binds to chondroitin sulfate A. The second thing that we've learned is that women become resistant

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to pregnancy malaria as they acquire antibodies that inhibit that binding interaction. And finally we've learned that anti-adhesion antibodies against these placental parasites are globally cross reactive, which suggests to us that there's one or maybe a few targets so that we don't know exactly sure how many right now, but a limited of targets for a vaccine. Next slide, please? So, that model of immunity informs us about what criteria we need to select the antigens for a vaccine. And—gotten a little bit off culture here—the first quality of the antigen is the vaccine candidate is a surface protein of placental parasites. The second thing is that the vaccine candidates will be recognized by sera from multigravid women but not by men. And the third thing is that the vaccine candidate will be targeted by protective antibodies. And specifically in my mind that probably means antibodies that inhibit the parasite from binding. And finally, the vaccine candidate is likely to bind to the placental receptor CSA, but it may be that there's other proteins there. We just have to identify which of the proteins is targeted by those anti-adhesion antibodies. And so the pathway to associate any of the antigens that we've identified with these functional genomic tools is, first of all, let's talk about what's on the upper right hand side; we use these functional genomic tools to identify what other specific proteins on the surface of

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placental parasites. And at the next level we then use sera from academic regions to find out which of these antigens is better recognized by multigravid women who have been exposed to malaria and similar parasites that is recognized by the first time mothers or men and separate from that finding, which of these antigens is really targeted by these anti-adhesion antibodies. And then those antigens that look most promising, we'll enlist antibodies against those to see if they react with placental parasites and our specific question there is, what's the diversity of placental parasites that they see? Is there going to be more than one specificity? And what we're looking for is the best antigen or combination of antigens that will achieve and broadly affect a vaccine. Next slide? So, the technologies that I'm going to talk about are microarrays. On your right, you can see a DNA microarray of malaria protozoites. Proteomics, on the left you can see a tandem as spectrogram from a tandem [inaudible] from what we call free studies. Looking at blood stage parasites that we collect from the field, and finally bioinformatics, these tools that you absolutely have to use to be able to master these technologies. Next slide? So, in the first instance, these are results of microarrays, studying field insolvency. And what Susan Francis has done is to label parasites that come from infected mothers, red, and she labeled parasites that come from infected

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children, green, and what you can see is that all of these genes out of the 5,400 or so that we studied with the microarrays are consistently expressed at a higher level in parasites that come from pregnant women. And on top of that list is a molecule that many of you are familiar with. It was first identified by Allisa Monte [misspelled?] as part of [inaudible] and large vintage groups. That's var2CSA; that's a vargie. But a number of other proteins that are actually single copy proteins. None of these are vargies and appear to be preferentially expressed by field dice that's collected from pregnant women rather than children. Next slide. So, then we have the list of genes that appear to be associated with placental parasites. Then we can go to our bio and paramedics tools and ask us, which of these have features of what we're looking for which is a protein that would be exported to the surface of the affected erythrocyte. And so some of the features that you would expect of such a protein are that they would have transmembrane membranes and what was recently described by Allen [inaudible] and [inaudible] the pexel sequences and you can see that all these genes that are encoding proteins that contain transmember domains and half of them have plexalcy sequences. So they have features that you would predict would be end proteins of interest on the surface. What I show in the last column is that all of these microarray

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studies are always confirmed because these functional genomics [inaudible] generate a hypothesis that are really providing the answers. And so those need to be confirmed with other tools that we use to quantitative PCR to confirm the level of transcription. And all of these are highly up-regulated and parasites come from pregnant women. Next slide? So our next tool that we call Freda is integrated into her studies is tandem aspect trometry [misspelled?] and what you see on the right panel is the maspect that Mascall [misspelled?] started with, which is called SBRI and on the left part of that is an HPLC, which you take a complex sample that comes from parasites, you digest it to make peptides, you separate the peptides on that HPLC, and that tandem aspect trometry [misspelled?] is able to tell you dozens or hundreds of proteins that are in that sample that you've collected. Now what McCall also does specifically is enrich for infected erythrocyte surface proteins used for using a technical approach that was invented by Jim Leach and Russell Howard a little over 20 years ago. So we're getting membrane fractions of infected red cells and then trying to determine what proteins are in those mixtures. Next slide, please? So, one of the limitations of masspect as opposed to microarrays is that there's a sensitivity issue. They're coming out with more sensitive mass spectrometers, but still we're not able to look

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for all thousands of proteins in any sample. Probably a limitation right now is hundreds whereas microarrays you really can get a sense for any gene whether it's being transcribed or not. But in—so three of the proteins that are there are not detected and therefore we cannot perform quantitative proteomics on that. Excuse me. But in the cases where we are able to detect peptides from those proteins, three out of four of them, including var2CSA as well as two single copy genes are expressed at a higher level in placental parasites than are expressed in parasites from children. Next slide, please? And then finally we can go to our genome data bases to get other features that are important for vaccine development and probably the most important is the degree of conservation. Because one of the parasite strategies for evading the immunity is to vary the sequence of its genes and what you can see with var2CSA is that it has 90 percent conservation with between the 3d prototype and the ganaian isolate that's been recently sequenced. And some of these other single copy genes have 100 percent. So, obviously the most appealing thing about an antigen would be that it's wholly conserved but then people start to ask: How can something that's targeted to protective immunity be wholly conserved? We don't have a final answer for that. But this is an important feature for a vaccine. Next slide, please? And then what you can do in a parasite that you

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can't do in a human host is really determine function. You can knock a gene out and do careful studies in a very rigorous manner to find out what the function is of any particular gene and this is the very nice work you can't see at the bottom it's from Artur Scherf's group where they've knocked out the var2CSA gene in the laboratory so that we're not able to regain binding to CSA suggesting that it has a role in parasite binding to the placental receptor. Next slide, please? And so—you can just run through those—so, this effort now is a multilaboratory initiative that's funded by the Gates Foundation to identify which antigen should be included in a vaccine and it looks as though we're down to a handful of antigens, most prominently var2CSA. The preferential express by placental parasites and I didn't show you the data but Lars and [inaudible] have already shown that there's parasitic recognition of var2CSA. So that is the farthest long in development. We expect that in the not too distant future there will be antigens going into testing for a pregnancy malaria vaccine. Next slide, please? The other example I wanted to give to you is a genetically attenuated pre-erythrocytic vaccine. This is based on the recent exciting work of Stefan Kappe from Seattle and Kai Matuschewski from Hydleburg [misspelled?], and the image on the left is from an editorial that Andy [inaudible] wrote about it in science. But basically we've know for 50 years that if you

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eratiated sporozoite forms of the parasites so that they would, to a certain degree, so that it was efficient to infect the liver, but the parasites were viable enough to affect the liver but not sufficiently viable to get into the blood stage. You could elicit to immunity that would prevent subsequent infection with wild-type parasites. And even though that model's been around for 50 years, there's still a lot of work and understanding with the immune basis for that and what the immune targets are. What Kai and Stefan accomplished were to knock out genes in rodent parasites and get them to rest in the liver in that fashion and then elicit immunity for subsequent challenge for wild-type rodent parasites. Next slide, please? So what is the ideal intinuated parasite vaccine that we're looking for? Well, first of all, it should arrest in the liver and not get to blood stage and cause infection. Ideally we would be able to use the minimal innoculum because making this vaccine will not be easy so the less the innoculum the easier it will be able to produce. We want immunity that induces—we want to induce immunity that blocks infection, or when you challenge with wild-type parasites, either in a challenged model or naturally, and very importantly we want to produce long-term immunity. And ideally that immunity would be boosted by naturally occurring infection. Next slide, please? So, the pathway to developing a genetically attenuating parasite

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vaccine is first of all to get something very difficult, which usually this isn't very difficult to get, is liver stage parasite material. But there have been advances recently, including these technologies that make it feasible to study liver stage parasites. And to understand what are the genes and proteins that are being expressed by liver staged parasites and you use that information to identify those which should be disrupted and to confirm that disruption of the genes and the parasite will arrest the parasite during liver staged development. And then in those animals that have received the attenuated parasites, we would immunize with these and challenge them with wild-type parasites to see if they are protective and then look at issues such as how long does immunity last and are there breakthrough infections in order to prioritize what genes should be knocked out and then to combine two gene deletions to make a safe and affective attenuated type vaccine. And very importantly these are studies in rodent and human parasites because there's so much more information you can get from the rodent studies, but obviously what you want to end up with is a human parasite vaccine. Next slide, please? Can you go back? So, these are DNA microarrays performed on infected HC04 cells and anybody that's familiar with microarrays will see that there's an enormous amount of background on these because there is so much more host material

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from the HC04 cells than there is parasite material. And this work has been a collaboration between [inaudible] in Thailand [inaudible] of Walter Reed and folks in my lab to try to define the scripto for the liver staged parasites. But with the use of these microarrays and bioinformatics [misspelled?] tools, we are able to identify probes that are detecting transcripts that are up-regulated in HC04 cells that contain parasites rather than other parasites, rather than other HC04 cells. Next slide, please? And taking those that appear to be specifically detected by liver stage parasites, and running them against all of the available expression data that's in plasma, db, and other resources, we're able to identify those genes that appear to be unique, at least between microarrays and biopharmatics tools, to liver staged parasites. And so this generates hypothesis that we then need to test. Next slide, please? And so, again our confirmatory tests is your quantitative VCR against liver stage parasites compared to other stages to find out that which, what kind of genes are these? Are they specific to liver stage? Here's an example of a gene that appears to increase steadily in it's transcription over time, during 72 hours of liver stage development and HC04 cells. Next slide, please? And here's a different liver stage specific gene that appears to be up-regulated immediately after invasion and to remain at a fairly high stage level during the

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first 72 hours of liver stage development. Next slide, please? And so using this strategy we have been able to go through all of the genes that we thought we were liver stage specific and to identify 38 genes that appear to be expressed as sporozoites in the liver stage, but not blood stage. Thirty-two genes that are expressed in liver stage and blood stage were not sporozoite stage and then 17 genes that are what we think are completely liver stage specific genes and so we do this work in parallel with Stefonsa who is the functional genetics on the road and studies and then this information goes to Stefan to then pursue these in the rodent model to see if these may be promising to create genetic attenuated vaccine. Next slide, please. And some of this information—this is exciting new work from Kai, Stefon, is how quickly now this information becomes an understanding of host/parasite interactions. One of the genes that Kai and Stefan identified has been up-regulated as a sporozoite—by deleting it you're able to arrest the parasite. It turns out enacts with the human protein apoA1 in which you see in the top three panels. On the left are the antibodies against the human protein apoA1, which co-localizes with the parasite protein uis4 in the middle panel and the coagulation is evident in the overlay. Next slide. By knocking out UIS4 in the parasite and you can see the parasite in the bottom middle panel. There's a small red palo there,

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you can see that there's not a corresponding apoA1 in that parasite because the gene has knocked out [inaudible] expressing UIS4 providing strong evidence that UIS4 is interacting with the human protein apoA1, which decorates the surface of the parasite vacuole. Next slide. And then what Kai and Stefan set out to do then is to get apoA1, knock out rodents, keep going, and to show that by challenging those versus wild-type rodents, in fact, apoA1 knockout, rodents are completely resistant to blood stage infection after sporozoite inoculation. But there's some data there if you keep tapping that. Can you push that forward a little? So, at two different sporozoite inocula, the apoA1 knockout rodents are completely resistant to challenged to wild-type sporozoites. Next slide. So, this path to genetically attenuated parasite vaccine is part of the grand challenge that was awarded to Sefon Cop and his collaborators that will develop hopefully over the next couple of years. Genetically attenuated plus modiocipro parasites that can be used to provide sterile immunity to people that receive them against a subsequent challenge. And I think I'll end there. Can I see the next line? Right. That's for the next section. So, thank you for your attention.

[Applause]

DOMINICK KWIATKOWSKI (OXFORD): Well, thank you. I

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feel like John [inaudible] because we seem to be debating whether the host genome is going to provide a solution to malaria. Well, first of all I think it's in question that—I was going to provide a solution that certainly there's no question that the parasite genome is going to be incredibly helpful. It's going to help us to understand mechanisms to drug resistance, help us to discover novel drug targets, vaccine targets, and as Patrick has just shown us, we can either make genetic care continued parasites. I know in humans we can't even make genetically attenuated humans, or at least we're not allowed to. So, the human sort of loses out coming to these things. Next slide, please? But I want to put it to you that all three genes are very important, that one of the key reasons they're all so important is that many of the things that interest us, particularly vaccinations in humans, many of the things exist as the host parasite interface, and natural diversity, at the host interface, both the parasite and the host, is crucial for understanding post parasite interactions. And I want to present to you the evidence the populations exposed to malaria, Harvard treasure and genetic information about critical host/parasite interaction. Next slide, please? So, let me just give you an example of a very simple paraseudoligal question. It's not really a human question. It's a parapseudiological [misspelled?] one. How do

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sporozoites get into red blood cells? And we know a certain amount of information about initial attachment events. We know, [misspelled?] for example, there's in the case of P viodox, there's a definitely binding protein that gets the parasites in. In a case of petrosum, is a more complex and redundant pathway. For example, epa175 binds and epa145 binds. Sounds very post pneumatical. Next slide, please? When all of these discoveries were made, as a result, of the analysis of human genetic variation, the Duffy binding protein gets few viadects into red blood cells, is discovered through analysis of the human genetic varium [misspelled?] [Inaudible], that the antigent. And certainly and analysis of glocoformis a positive red blood cells, or people who lack sublike of foreign A, were vital clues in defining the EVA one in parasite antigen and similarly, that like a foreign C, deletion of variance red cells that act like a foreign C, were great clues as to how epa went 40 work. So, next slide, please? So, in the case of that particular biological phenomenon, human genetics has been very useful. And I want to deal with Dorothy and [inaudible] in detail because it provides a paradigm for how huge genetic discoveries may lead to practical solutions. In this case, it began with an epidemiological observation. The P vivax infection is rare in Sub-Saharan Africa. That led on to human genetic discovery. Made by Lue Miller [misspelled?] and

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colleagues, that most Africans with the Duffy antigen and the P vivax require the Duffy antigen to invade the cells. That was the genetic mechanism. That led on to biochemistry and molecular biology again, largely in Lue Miller's [misspelled?] lab particularly with [inaudible] and colleagues, sharing that the Duffy antigen, I'm sorry, using the Duffy antigen, has a biochemical and electrical biological tool to identify the parasite binding [inaudible]. Which was Duffy binding protein. And then now, from that work, it's been pushed into the back seat. Development pipeline and Duffy binding protein is one of the vaccine candidate for P vivax vaccine. So, that's a sort of trail of events that has human genetics at the top but pushes it through into the vaccine development pipeline. I haven't asked Patrick Duffy if he's related to the Duffy antigen and he claims not, and so, I think actually, it's because he's ashamed of his association who's made such a strong case for human genetics being used to invite practical solutions to malaria. Next slide, please? Now, I want to go on to suggest that, quite aside from finding how parasites get into red cells, if we just look at the clinical determinacy of protective immunity, we do well to start looking at human genetics. The strongest known determinant to severe malaria is a human genetic factor. So, if you are a heterozygote for the human [inaudible], you essentially get 90 percent protection

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against severe malaria wherever you live. And we don't know any other effect that's as strong as that. Bednets, as far as we know, aren't quite as strong as that in most communities. And certainly any vaccine that's as strong as that and we don't know any parasite genomics as strong as that. Next slide, please? And, of course, the reason why the human level [inaudible] is at such high frequency in much of Sub-Saharan Africa is because it's being selected by malaria. Next slide, please? And indeed malaria is the strongest known force for [inaudible] selection in the recent history of the human genome as well as sickle there are other variance of [inaudible] structure like [inaudible] there are variance [inaudible] and a variety of other red cell polymorphisms. They have all risen in frequency in human populations because they provide protection against malaria. Now, you'll see that in this list here, it's all erythrocyte polymorphisms, but I think that historically because the erythrocyte is quite an easy cell to study. You can easily see variance of red cells just by looking down a microscope at some blood. And it's been a regular work force for molecular biology and molecular genetics. Of course, it's been historically much more difficult to study immune cells, particularly when its immune cells happen to be living in the deep recesses of the spleen. But, it's highly likely that malaria has exerted such pressure

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from the immune system and [inaudible] approach has allowed us to make a comprehensive analysis of genetic variation in the immune system. Next slide, please? Now, you might say that with all those red cell polymorphisms I just showed you, pretty well everything that is important has already been discovered. Particularly, I'm always amazed when people make that argument to me because it seems incredible that having to select a few genes instead of looking at the 30,000 or so human genes, we'd have discovered everything. But, here is clear evidence that is not the case. A recent study from [inaudible] and Mark Williams in [inaudible] Kenya estimated that host genetic factors represented about a quarter of all the observed variation in incidence of both mild malaria and severe malaria in that population. About the same, in fact, as variation in household. And you might expect your household would matter. You know, did your mum give you [inaudible] or do you sleep on bednets? The host genetic effect is just as important as that. Importantly, the sickle effect, account for only two percent of the variation. So, there's a lot of other affects yet to be discovered. Next slide, please? Now, before we get on to sort of any detailed analysis of children with severe malaria, and that's going to be the main [inaudible]. We can learn something just by simple analysis of populations. A recently published study looked at one and a half million sniffs in

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three population groups, found that 20 percent of the sniffs, by the way a sniff is a single-type polymorphism. It's a [inaudible] that exists in the very base of the genome. This study found that 20 percent of those variances existed in only one of the major population groups. And when that happened, it was most likely to occur in Africans. In other words, Africa is the continent with greatest genetic diversity. And we can ask how much of that diversity has arisen through selected pressure of malaria. Of course, there are other very good reasons for that increased diversity in Africa. Africa is the cradle of mankind and many of the European and Asian populations have migrated west of Africa. So, it's hardly surprising that most of the [inaudible] diversity exists within Africa itself. But, it may very well be that a significant part of that variation has arisen through the pressure of infectious diseases, especially malaria. Next slide? I'm afraid you can't see the bottom of this slide, but it refers to a very important recent publication published within the last month that I hope some of you will look at. There's a major publication from the [inaudible] Contortion, which is the first comprehensive of human genetic diversity at the genomic level. The contortion, which included groups in many different countries, looked a million single nuclear type polymorphisms as of the ten million polymorphisms that are now know to exist

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throughout the human genome. And they compared the frequencies of [inaudible] and the frequency of [inaudible] between three major population groups: a group from [inaudible] in West Africa, a population of European ancestry from the states, and also a couple of Asian populations. And what I'm showing you here, is that if you compare just the frequencies of these polymorphisms between different populations, what is immediately apparent is that certain variance are completely different between different populations. Let me give you an example of what I mean. Let's just say there was a position in your genome where you could either be a G or you could have a C; that would be a single type polymorphism. What we're saying in this example is that everyone in Africa has a G; no one has a C, and everyone in Europe has it the other way around. That's extreme differentiation. Now, until a few months ago, we only knew of one example of that in the whole of human populations, and that was the Duffy antigen. The Duffy antigen is essentially very close to 100 percent frequency in Sub-Saharan Africa, a very close to zero percent frequency in Europe. But here's a whole bunch of other things that just come out of a random analysis of other genes that have that same extreme divergence. Now, of course they may have risen by chance locally, maybe enhanced by other factors, but that's an obvious candidate gene to look at to see if they contain any malaria

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protective variance. A cue point here is that [inaudible] is thought to become a major killer only about 10,000 years ago. In other words, subsequent to the differentiation of major human populations. So, if [inaudible] had been a major source in Africa over those last 10,000 years, the [inaudible] might have risen in Africa but might not have represented Europe because Europeans had left before then. And then, I know we're getting too technical here, but another sort of analysis that we're particularly interested in our lab, are these sort of large data sets. By using sickle [inaudible] of recent positive selection. For lack of time, I'm not going to go into that, but I'd be happy to talk more about it later to anyone who's interested. But the bottom line is that that also reveals some other animals that have recently been selected in Africa, which would be top candidates for us to look at as top malaria protected genes. And you'll be interested to see that the list that came off a random search of the genome included the globan [misspelled?] [Inaudible] that contains the sickle [inaudible] also included Z36, a gene which has been associated with susceptibility to malaria. Next slide, please? So what are the questions we'd like to answer? We'd like to know, what are the mechanisms that clear parasites from the blood? I want to know the immune systems that's protecting its malaria. And the road block to—and, of course, many of us have been trying

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to address those questions for 20 years or more and we all have frustrations and we all feel that we've made some good discoveries as well—but there are some road blocks to conventional investigation. Firstly, in our conventional ways of looking for protective immunity, there are thousands of molecules that we know nothing about. Many of which may be quite important for immunity. That we can't get to the site of the action. When we saw the blood from a child with severe malaria, we know that that's sort of interesting but it would be even more interesting if we could get into the spleen or the brain. And also, while we find that things change in the blood, we find an association with a particular molecule. [Inaudible] is associated with severe malaria. So it's very difficult to know whether that association represents a cause and effect, or, alternatively, is to [inaudible] phenomenon. It just goes up. As a sort of just falling off from other things. Next slide, please? Well, one way we can try to grapple the issue of novelty is to do like gene expression profiling. And this is very similar to the sort of thing that Patrick's showing you where we do microarray analysis of thousands of different genes, and we ask which of them come up in different groups of different people. This is a microarray analysis that Mike Griffiths [misspelled?] with the collegiate group that identifies a whole bunch of genes that come up in

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the blood that are associated with acute malaria, the whole bunch that seem to rather nonspecifically associated with immune responses to a variety of infections and so on. Next slide, please? The problem with the analysis that I've just shown you is that although it gets novelty, it doesn't really get into the problem of corzality [misspelled?]. It would be difficult to know whether those genes are going up and going down in the previous slide are corzal [misspelled?] to the severity or they're just anti-phenomena. And this is where genetics is useful. Let me be sure you know what I mean. If you think that protein X may play a critical role in protective immunity for severe malaria, you examine the level of protein X or the RNA or protein X in the blood of children with malaria and see if it's associated with mild or severe disease. The problem is, that if find an association, you're not entirely sure it's an anti-phenomenon or not, and secondly, if you don't find an association, it might just be because the effect is working somewhere like the spleen and so you're not seeing it when you saw the peripheral blood. Next line, please? So, genetics helps in this way but, if there happens to be variation in the gene that interest us, and if we happen to know that one of the variants of the gene was also in high production and the other was also in low production, then we could do a very large epidemiologic study and see which of

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those variants is associated with severe malaria. And even if the gene is only expressed in the spleen, you may still find an association. And you know it must be corzal [misspelled?] because you know the person had the gene before the disease happened. The disease of course in variance be present so the variant must be have had something to do with the disease being present. So you can determine the direction of corzality [misspelled?]. Next slide, please? So, it's some very exciting times to be doing genetic association and analysis of severe malaria, the genome has thousands of genes along their function, some of them may be very critical for immunity. We being to understand the extent of human genetic diversity, [inaudible] that mutate technologies through [inaudible] typing. We can now type half a million synopsis in a single aspect. And we have new methods of identifying which snips cause genes to be up-regulated or down-regulated [inaudible] is a main interest in our lab. Next slide, please? So, what we need to do now is extremely large medical association studies of severe malaria, to try to find variance that our association for susceptibility for resistance. The trouble is that we're not expecting these variants to have very strong effects. Because there are many of them, and because of so many different effects, the effect of any single varium may be quite modest. The second problem, is if you were to look at a

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million snips across the genome, and that's how [inaudible] in the next few years. We're to do a lot of corrections for multiple comparisons, and that requires a sum of a very large size. We reckon we need about 8,000 cases of the severe malaria and 8,000 population controls to do this study properly. Next slide, please? And so, it follows and human genetics analysis has to involve African researches and African communities for many different reasons. But not least because of the very large sum of [inaudible] required. And the next part of our slide shows—should I just move onto this very briefly. I'm getting signals [inaudible] involving African researchers, but I will fast forward so I can see Francine waving at me. So, very quickly, next slide, please? Well, I'm going to fast forward again. That's all right. Next slide, please? Okay. In the vital few slides, I just want to talk about the importance of all the African researchers in this and building capacity. And just before I get to that, I feel I've got to entrance the catastrophe of it, and just say that genetic association analysis on a large scale, sort of just described to you, [inaudible] is much easier to do with human genetics than with the parasite. And the reasons for that are of course we don't know the contribution of the parasite genetic factors of the disease. We know that the human genetic has a passive genetic component, that this problem is not of

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infection, when you saw that the blood from of the child with severe malaria is difficult to know whether—you certainly know that that blood contains the DNA for the parasite that's causing the severe malaria, but you it certainly contains the DNA for perseverance malaria, but you know it surely contains the DNA for the parasite that is causing the severe malaria because it may be a [inaudible] infection. But finally, and I'm not going to dwell on this, there are complex matters of understanding compilation structure which we understand is fatal among human populations. We don't [inaudible] and that makes it enough to be quite difficult in the case of parasite genetics. Next slide, please? Next slide, please? Next slide, please? So, I just want to say that human genetic association analysis at this level is possible. These are some of our collaborators that we're working with and our greatest grand challenge and you see that already we've collected over 8,000 cases of severe malaria in studies that have been going on for many years. And we're hoping to collect a great deal more than that over the next two or three years. Next slide, please? We're working, as part of our [inaudible] based challenge on this in 20 countries, 40 of them malaria in [inaudible] and 10 of them are in Africa and some of them are listed there. Next slide, please? And, next slide, please? So, our objectives include over the global data sharing network

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for global sharing epidemiology malaria, collecting DNA data from different individuals with different malaria fena [misspelled?] DNA types, charactering different variation in the malaria demo populations. Identify different variances that protect against malaria. These large multi-symptom studies. And defining illogical mechanisms of genetic variance that protect against malaria. Next slide, please? So, large scale of the epidemiologic studies with massive [inaudible] with human variation, and what they need to type, generally to determine the [inaudible] types. That's to say, two-thirds of a million snips tried in several thousand individuals in the next couple of years. And then, from that, hoping to pick up genes that seem to give ultimate protection, and then try to understand their functional role, and then translate that into understanding that protective immunity. Next slide, please? Next slide? What we're trying to do is build a global data sharing community with [inaudible] and when databases are critical to our process. We have multiple study sites that are running their own studies. They're contributing DNA in the essential field of data from infected individuals. And they go into a contortion geo [misspelled?] sitting effort that generates important associations and publishes it as soon as transparently as possible. And then the genetic data goes back to the state sites for investigator analysis, for detailed

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analysis, that will address specific questions be addressed at each of the independent study sites. So, there's a central consortial component that also all these genetic data goes to feed individual analysis at each individual site. Next slide, please? So, one of the key things we're trying to now is to build capacity of human genome research in malaria in other countries, particularly in Africa, and that involves agreeing in implementing rigorous case definitions, particularly in severe malaria [inaudible]; therefore, when these web tools are discussed to empower our collaborators in malaria and other countries, unlike [inaudible] data, develop the best practices for consent, data sharing, and intellectual competency, and such a huge issue here, on intellectual property and data sharing. Understanding how to work collectively without eroding [inaudible] individual sites. And engage in the communities themselves because they're genome that we're studying. Thank you very much.

[Applause]

FRANCINE NTOUMI, PH.D. (LAMBARENE): Thank you very much. Dominican, that's a summit we had started in February last session and last discretion and we will would like to talk about malaria research in Africa. Dominick presented his point of view, unfortunately not fastly, but anyway, we can enter what so for what the junior senses what do you think about this

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new feed of research? Do you think that you are involving and have you an interest in work on this plan of studies. And please, would you give us your point of view about these new technologies? Any Comment from the floor? There's no young scientist that to be trained by informatics and in these new technologies? No? [Inaudible - French] Yes?

MALE SPEAKER: I find trait that you mentioned that the consent to study subjects in multiple subjects was a huge issue. Were you particularly defined to genetics, and therefore find strong risk factors that should in fact be fed back to study subjects and how is that final situation being tackled?

PATRICK DUFFY, M.D. (SEATTLE): The question was whether there—if we get genetic information on risk factors, will we feed that information back to subjects? This is being discussed a great deal, not just in the malaria community, but in the whole human genetic analysis community, and I think the general agreement is that that is wrong to do that. There has to be an understanding that when people contribute their data for research there's no possibility of it being abused in any way and that involves complete anonymity and confidentiality. Of course, once genetic data flows back to the individuals, it can easily be made of [inaudible]. For example, if you think you're doing a service to someone by telling them that they've

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got hemoglobin sickle cell disease, you know most of you would be doing a dis-service because one of them doesn't carry the [inaudible], maybe evident that [inaudible]. So there are lots of issues about feeding data of this sort back. Instead, we're needing to a slightly different solution, which is that risk factors were identified will be published and if there are grounds, are really good grounds for communities knowing who is at risk, that would be set up as a separate service. So, in other words, if there was a high incidence of some polio, where, knowing the results, could improve the health of the population, then that should be provided as an independent facility after the study's completed. Does that answer you question?

FRANCINE NTOUMI, PH.D. (LAMBARENE): Okay. Is there any other question to our discussions? No? No? [inaudible]

MALE SPEAKER 2: [Inaudible] a comment and a question. First of all I would say that these new technologies are coming. They're welcome. That's the only choice we have. Because there will here more and more involved here so [inaudible] agriculture or whatever. The comment I wanted to make is that I think that become now important in our own faculties as opposed to adapt the syllabus for teaching biology and biology co-sciences. We've got to continue this as usual and expect that the [inaudible] scientists, young scientists,

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are would be able to contribute. They can be very group receptive of to these new technologies, just like we used the radio, the television, and sometimes the [inaudible] without knowing how these things work. But if they remain a problem to affect technology, then I think we have to look at it [inaudible] and make a necessary adjustments. Because it's well and fine between [inaudible] using a ready need [inaudible] but sometimes these things have limits. And that's where the real confrontation begins, when you can mortify adopted or even invent something [inaudible] Thank you.

[Applause]

PATRICK DUFFY, M.D. (SEATTLE): So, I won't go back to my slides. But I do have a couple of slides at the end as Dominick talked about the grand challenge that we received. I was going to build infrastructure where we work which is Tanzania. We have a very successful training program that's been sponsored by Fogerty [misspelled?] to train young scientists. So really to make this a reality in Africa you need the facilities and you need the human resources. So, our approach has been to develop a genome of science center at [inaudible] University and it's also my awareness that there's going to be a facility up at Illray [misspelled?] which will be focused on genome sciences as well. So, there's already connections between those facilities. And we have—there's no

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bioinformatics importees available right now in East Africa, so to accomplish our research projects, we have actually gotten three computer science graduates from East Africa who will now become part of the bioinformatics training program, do the research as part of the program, and hopefully will create a corodex [inaudible] for science and they will be able to build future studies of science on. So, that's been our approach with TK [inaudible] and [inaudible] and [inaudible] University in respect to Tanzania, and that was the plan to develop those capabilities there.

MALE SPEAKER 2: Yes, thank you very much for your point. It's a very good one. And as Patrick said, we, too, try to build a capacity in all cases across a network and it's largely focused on capacity for clinical epidemiologists as well as bioinformatics and also antigen technologies. But another thing that's emerged in terms as getting consent from communities, is that as we all know, when you ask for consent to do research, often communities don't understand what you mean by research. And certainly when you ask for consent for research on genes, they certainly don't know what you mean by genes. So, we acquired interest in possibility that as part of our process in getting valuable consent, we can start to initiate some sort of community education programs. We haven't made any great progress with that yet, but with falling on

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largely from suggestions that came from other [inaudible] where they've been working with linguists and anthropologists to try to explain things to communities and what has been exciting to us is the possibility that we could provide teaching materials to primary schools, which could be distributed quite freely in communities where we work. That doesn't necessarily ensure that individual subject has read them, but at least it starts to improve awareness in the community as a whole.

FEMALE SPEAKER: My question is about the human genome projects and what I was wondering was, how much of this involves education? You said something here about genetics and something about race, differences, and things like that. The press does get sensors of those, I think. And I don't know which you are doing in Africa to ensure that there as such does not get even messed up by getting very bad reporting of participation of your data. And how will you protect yourself? I mean, you said something about PR in Africa because these issues do get sensitive by the presses.

PATRICK DUFFY, M.D. (SEATTLE): So, can I be sure I understand what you are asking? This raises the issue of race and they're very sensitive issues and how will we deal with them. Did I—yes?

FEMALE SPEAKER: Especially with the media because some of this things when they are unclear can [inaudible] and you

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have—have you started putting people in place to get in touch, that the media can get in touch with? PR, there are people for your research groups so their issues are right based on someone who can give them correct information because the press now is all over the place and want to know what's happening. They're likely to misinterpret things if someone is not there to explain these things in Africa.

PATRICK DUFFY, M.D. (SEATTLE): Yes. Well thank you for that question. It's a very important one indeed. I think the first point to make just so everyone is aware of this, is that what is remarkable about human genetic diversity and we now understand this much better than we did a year or two ago, is how similar different people are, not how different they are. If you took any single village anywhere in the world, well, not every single village, but about 99.9 percent of the villages, you find more genetic diversity in that village than you find the differences that exist between any major population groups. Most of the human genetic variation is actually within us. Just in a straight up point, if I took any of you now and I took the genome that your mother gave you and genome that your father gave you and lined the two of them up, I'd find about a million differences, on the average. So, I think the first thing that we're learning is that race is a very blurred concept. But still your point is a very good one

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and I don't think we've got completely solid answers for it, but we're working toward them. In the Indian International Hazmat Organization, which is this large survey essentially of differences between you Indian groups, Africa, and Asia. A huge amount of community consultation went into it and a lot of ethical and legal agreements were reached between institutions and individual communities. I think it could be fairly said that all the people involved, all of the stake holders involved, including the communities, were happy with that. And I think that sets for us a sort of reference point. Perhaps how you do approach these issues. They're certainly not to be taken lightly and they represent an additional reason why the process of consent is extremely important because the communities need to understand that it does touch on the issues of race. Does that sort of address your question? Partly. Okay, a person can talk about it more later.

FRANCINE NTOUMI, PH.D. (LAMBARENE): Okay. So that was the last question. I would like to thank you very much for your participation and another thing, Patrick and Dominick for their wonderful talks and thank you. Have a nice evening.

[Applause]

[END RECORDING]

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