

**Current Controversies: Parasites—Are All Malaria Parasites
Created Equal?
The Fourth MIM Pan-African Malaria Conference, Yaoundé,
Cameroon
November 15, 2005**

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[START RECORDING]

KAREN DAY, Ph.D. (New York): . . .to the next slide, please. Many of you have heard me speak before. I have stolen this Kandinsky painting, and I think the point I wanted to make here is we have lots of genetic diversity, but also when we all look at the same painting, sometimes we see different things. But a lot of work done by a group in Edenberg [misspelled?] Danny Conway, my group, and many people in France and Africa have shown that there are many distinct plasmodium falciparum genomes based on microsatellite markers, based on antigenic markers, and also phenotypic markers. If I have the next slide, please?

Many of us would believe that this genetic diversity has evolved to allow super infection, to allow the human host to become infected again and again and again. If I could have the next slide, please.

This is showing very clearly here, where you would see a longitudinal study of a child for 60 days. This child happens to be on the North Coast of New Guinea, but I think the same point could be made about children in Africa at least with respect to falciparum genotypes, but here we sort of see through the course of 60 days, this child was infected with nine different MSP-2 genotypes.

So we have a very crude way of just saying children are

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infected with a lot of genotypes, and this child is also infected with four heterodimer X [misspelled?] genotypes. So the general belief would be that diversity has evolved to allow a host to be super infected. If I could have the next slide please.

The diversity that we see which is quite extraordinary, we believe to have arisen because sex is obligatory part of the malaria life cycle, and this is very different to bacteria in viruses that are built into the basic lifecycle within the mosquito host. There is the capacity for novel genomes to be created during sexual recombination in the mosquito vector. If I could have the next slide, please.

Many of you would know that as the transmission systems in malaria endemic areas are characterized by individuals carrying multiple genotypes. In low transmission areas you might get people carrying zero or one genotypes, but in endemic areas, highly endemic areas, people are carrying two or more genotypes, so there is plenty of possibilities for recombination to occur in the mosquito midgut.

And by that I mean, if you have a red clone and a blue clone and you have the male and female gametes, you can have fertilization between the red males and females and the blue males and females or between each other, and that would be a cross fertilization event.

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And over the last 10 or so years, David Monaco's [misspelled?] group and my group, in particular Hanz Babaker [misspelled?] Lisa Ramper Cartwright [misspelled?] and we pulled—have gone out and actually measured how many matings are cross matings in nature, and 60 percent of matings in Tanzania would be cross matings, 10 percent of matings in PNG are across matings, so there is plenty of possibility for creating more novel genomes in sexual recombination in the mosquito vector. If I could have the next slide, please?

The power of sexual recombination in the mosquito vector, I think, is seen very, very clearly by the evidence that is now coming out about the emergence of drug resistance in Africa, and a book by Kelly Roker [misspelled?] and others has shown very clearly that pyramethamine resistance has occurred via selective sweep on chromosome IV, and you can see that a region of the genome that is present in Thai parasites has made it's way, if you like, via sexual recombination into African parasites.

So the parasites can use the ability to undergo sexual recombination to generate extraordinary diversity and also to spread advantageous traits very easily. If I could have the next slide.

Over the last 10 years, we have come to understand quite clearly that the origin of falciparum is in Africa, and

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that we see the most diversity of plasmodium falciparum in Africa, and this is seen quite clearly here—at least when you look at microsatellite markers, one lane can cause on parasite.

And we are comparing a parasite population in Zaire and Columbia, and we can see just extraordinary diversity in the African population—very limited diversity in the Columbian population. And what we have come to understand in that previous slide is that we are seeing linkage equilibrium in the African population. We are seeing that sexual recombination is jumbling up the haplotypes, if you like, whereas in the Columbia population we are seeing clonal parasite populations by and large in the South American system.

So there is a very big difference in parasites from African and parasites from South America. Parasites from South American tend to be spread in commonly, but parasites in Africa are constantly recombining through this sexual mechanism that I have described for you.

A number of us have been concentrating on trying to understand the evolution of falciparum, and while we know it is something falciparum-like has been coexisting with our hominid ancestors for something like 5 to 7 million years, we have come up with two models of evolution of plasmodium falciparum.

And one is that many ancient lineages could have effectively persisted until today, and that we would see both

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the large amount of diversity we see in say African populations today because the parasite is old and there are lots of lineages, or alternatively, a model that was put forward by Francisco Iala [misspelled?], and indeed, myself and colleagues at Harvard was that potentially there could be a recent common ancestor to extend falciparum and that there had been an involvement that had occurred and the diversification from that.

I think now the work Zing Won Su's group, Deirdre Joy and colleagues there at NIH has shown pretty nicely that five mitochondrial lineages appear to have persisted and diversified giving the parasite population that we see today, and there was extraordinary expansion of the parasite population around 10,000 years ago during the dawn of agriculture.

And the debate that we had at the time whether there was an ancient common ancestor in this—and this is a recent common ancestor model—where it was resolved by work of the NIH group who showed very nicely that 80 percent of the diversity in the genome lies in 20 percent of the genes.

And that the reason we were getting quite different models of evolution within different part of the genome was simply because some parts were monomorphic and certain parts of the genome were highly diverse. If I could have the next slide.

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Parasites in Africa, Asia, and South America are also different by virtue of microsatellite haplotypes. We understand very clearly from some work of Tim Anderson's that African parasites are very related to each other. We see no genetic differentiation between microsatellite haplotypes in the African population, but we do see that these parasites are different compared to Thai parasites in South American parasites.

And this probably very much relates to interactions with vector and also from founder effects particularly in the South American parasite population where we believe the population is fairly recent, only occurring there in the last 500 years. If I can have the next slide.

Parasites differ also or they are not equal in terms of their ability to transmit through the major vectors of malaria and this was pointed out as early as 1935 by James and also in the 1970s by Mario Koltsy [misspelled?] who did very elegant work looking at whether the tropical strains of plasmodium falciparum could be transmitted through the European vector anopheles, that Tracar [misspelled?] who showed very clearly that they couldn't be, and therefore the possibility of reintroduction of falciparum from the tropical regions into European where malaria controlling occurred was not likely.

So this strong, local adaptation with respect to the

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vector, so all parasites are not equal. We have done experiments recently—and this is work by Jen Heden [misspelled?]-who looked at Thai parasites and whether they transmit easily through *Anopheles gambiae* the African vector, and they transmit much less well compared to African strains; so all parasites are not equal with respect to vector transmission. If I could have the next slide.

Okay. Now just to spend a few minutes on strain theory to get into the meat of the debate. About 11 years ago, Senetro Guptor [misspelled?] and I were working on trying to figure out how to calculate the basic reproductive weight from malaria, and we developed a strain theory to think about the malaria transmission system.

And it came out of looking at age-seroprevalence curve where you look at the proportion of children with antibodies to malaria and you compare this to measles, and immediately you would think malaria is much more transmissible than measles because your risk of infection is much higher. Your average age at first infection with malaria is much lower than it is with measles, say in a developing country.

So from this curve, we started to think well what happens if you start thinking about the malaria transmission system in terms of strains or different types of malaria? Then you might see that the transmissibility of an individual

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parasite strain might be much lower, down here.

And this had very obvious implications for malaria vaccination because if you had 100 percent transmission-blocking vaccine with malaria, you would calculate the proportion of the population to be immunized to block transmission by an understanding of the transmissibility of the organism.

So the economists found our ideas very interesting. The malaria community hated them for all kinds of reasons. The essence of the strain theory really was to say that malaria wasn't one thing. It could be many strains, so essentially what we were saying was that all antigens were not created equal, and that some antigens were more important than others in terms of immunity. If I could have the next slide, please.

So basically we proposed an antigenic hierarchy with the antigenic targets of immunity that affect transmission are the most important, and this really was the essence of strain theory from the parasite genetics point of view. If I could have the next slide, please.

The antigen we focused on, which we didn't have this name for them, but essentially was the plasmodium falciparum erythrocyte membrane protein I, and we at that time had shown—and others of course had shown—that it was on the surface of the trophozoite-infected cell and also worked by micro—and now

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Collin Sutherland has shown—that it is expressed on the early gametocyte. And we know that there are 60 variants per isolate and that each isolate has possibly a different repertoire of variance. If I could have the next slide, please.

We believe that PMFP-I was the important antigen to focus on in terms of thinking about strains because antigenic variation facilitates transmission success. It does this by promoting persistence to allow transmission, and I think in sub-Saharan Africa particularly work by Hanz Sabadacker [misspelled?] showed very nicely that clones need to persist for a 10-month dry season in order to be transmitted in the next rainy season.

Antigenic variation also promotes gametocytogenesis by being directly involved in the gametocyte development, but also by virtue of the fact that the production of gametocytes requires that you have high density asexual parasitemias.

And so any immunity that regulated asexual density would inhibit the production of gametocytes but also if you had different repertoires of var genes in parasites, then effectively, antigenic variation would allow super infection to occur. If I could have the next slide, please.

So to get down to the meat of where we are at now and to perhaps give some answers in terms of strain theory implications, for the past five years, a group of us with some

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colleagues in the field have been trying to sort out the population genetics of var genes from a very basic population by all gene point of view, not taking any notions about relevant or relatedness to disease state but just doing random sampling of the parasite population.

We looked at parasites in New Guinea and also parasites in Dubois. If I could have the next slide, please.

We looked at the DBL alpha region of the gene just simply for as all parasites have this region—I am sorry—all var genes have this particular part of the gene and we PCR'd from a range of isolates, DBL alpha regions in a random sampling protocol. If I could have the next slide, please.

The results of that study are shown here, and here you have PNG repertoires and here you have the bond repertoires, which we are comparing. And if you look where an isolate is shown by a track and you can see different var genes that have been picked up in essence in each individual isolate.

And the first thing that we point out is in the PNG parasite population, just sampling a local village, 30 isolates; we picked up 149 DBL alpha sequences. In contrast in the bond population just looking again at a local village, we picked up in 29 isolates that there 666 different DBL alpha regions.

And when you look at how they are organized within

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individual isolates, within the PNG population, we found no two repertoires to be the same within the local African population; in the bond we found no two repertoires to be the same. So the point about var repertoires being so different that a host could be super infected, I think, is a plausible argument based on these data here.

Interestingly, we found very few genes in both populations to be common to all isolates. We found rare types that appear only once, these are the black areas that are shown here, and you can see there were more unique or rare types in the African population; that is, unique sequences. Now where you see the colors the same color across would indicate that an isolate—two different isolates might have that same DBL alpha sequence, but you can see that is not occurring very commonly. So essentially we are seeing extraordinary levels of diversity.

Now an extension, or an additional part of the strain theory was put forward by Senetro Guptor who said that she developed a theoretical argument that it would be plausible to have groups of var genes linked if they gave an immunological advantage an immunoevasion advantage. And I think these data would point to the fact that we are not seeing these at all. If I could have the next slide.

I think what these data would point to that if we are going to hold to PFMP-1 being the strain-determining molecule,

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we might have to start thinking about a strain at the level of the individual var gene. The number of strains is the number of var genes encoding unique serological variants. If I could have the next slide.

So I will conclude by saying that as far as I am concerned, all parasites are not created equal. There's polymorphism in drug resistance, polymorphism in single copy genes. There's local adaptation of anopheles vectors, which is encoded within the genome. There is also geographic differentiation, and I think very importantly, this diversity in var gene repertoires and we've got an enormous amount of work to do to really go on and sort out these genes, and better understand the population biology of these important immunoevasion genes.

And I will finish with Charles Darwin and say that I am sure he would agree with me. Thank you. [Laughter]
[Applause] I also want to say David is looking splendid, you know, in a tie here. [Laughter]

DAVID ARNOT, Ph.D. {Edinburgh): Thank you, Karen. And remember today that everyone is required to wear a jacket and tie. [Laughter] Okay. Can we have the next presentation up, please. Thanks. Okay. As to debate the point are all parasites created equal? My answer would essentially—would be in essence, yes. If I could have my next slide, please.

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For example of extreme inequality in malaria parasites, we need to go to the theory of clonal plasmodium falciparum populations of Lurch and Iala, and a quotation from a very well-written paper, "Insects Vector, Parasites Say No," and Professor Iala puts forward the following rational.

"Within clonal organisms, the search for vaccines and drugs is more likely to be successful if preceded by identification of clonal lineages targeting those that are more pathogenic or ubiquitous," and this is clearly a statement that I think we could all agree with and it is well put.

From what he presumed to say was that in clonal organisms, the persistent entity is the clonal lineage, and genetic diversity of species can be captured only by extensive sampling of distinct lineages.

Carrying this a further step and adding into the mix an analysis of the sequences of a number of plasmodium falciparum antigens who show the striking phenomena that is a possibility of nonsynonymous genetic substitutions in the parasite.

Richard and Iala put forward the so-called "Malaria's Eve" hypothesis that comes a little bit in terms of recent common ancestor origin of plasmodium falciparum populations. I think that there are reasons—which they are beyond the scope of this debate—to disagree with that hypothesis which relate to the origin of the possibility of the nonsynonymous

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substitutions, but that's a subject for another debate. Can we go back to that slide, please.

And put here—and I think most extremely Steven Rich did eloquently put it, and I think crystallizing the argument—a clonal population structure is consistent with physiological sexuality as is required in plasmodium to complete the lifecycle, but excludes the prevalence of genetic sexuality, i.e., recombination between genetically heterogenous haplotypes.

So we have an extreme position here on the fact that plasmodium falciparum populations are not equal because they are composed of clonally dividing lineages that rarely—if ever—indulge in sexual recombination. Can we have the next slide, please.

Now, to clear one point of controlled [inaudible] that should be swept out of the way. If we wish to clone drug-resistant isolates such as we all frequently take from patient populations drug-resistant strains, then we are free to do so, but we would get clarity and analytical insight if we instead called such isolates by the more accurate term of "drug-resistant mutant." And in population genetic terms, selection for resistant mutants would transiently decrease population variation.

The period of how long they will do that will depend on

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how much recombination is going on in clonal organisms. In true clonal lineages, it would take a great deal of time before populations and variations redeemed, and rapidly recombining organisms are organisms that would not take very long.

Now outcome data on the population genetics of *plasmodium falciparum* is compatible with clonality and clonal searing of *plasmodium falciparum*. Quoting again from *Malaria's Eve*, the Rutch [misspelled] et al. Paper, which as Professor Liz alluded to, she also lent her considerable weight to a series of publication postulating the recent common ancestors, Mr. Benz tried to sweep some of the affect in our chromosomes and it could happen if the population structure of *plasmodium* were effectively clonal; however, this has not happened.

Selective sweeps are associated with fully immuno [inaudible] resistance that are confined to 70 kilopieces of DNA around before they are able reduct these genes. High-density microsatellite chromosome marking shows that recombination is now [inaudible] quickly.

This is completely incompatible with effective population clarity [virus database has been updated] of *plasmodium falciparum* and some very interesting work has been carried out by the work of Kelly Roker's [misspelled?] group at Antaton [misspelled?] School of Tropical Medicine and I refer you to a recent publication of theirs—and an excellent

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publication.

So can strains exist in a genetically sexual plasmodium falciparum? I think we can effectively discourage the idea that there no genetic sexuality in plasmodium falciparum, which was always a rather extreme position, all be it was held by eminent population geneticists who, sadly, can write very convincing paper.

The evidence against clonal lineages of plasmodium falciparum in all except fungal effects, the evidence is strong against certain American data, which Professor Day alluded to, is clearly something of a fungal effect in the low level intensity and transmission in the South American forest.

So if recombination is frequent all the proportions of transmission intensity, can strains exist in plasmodium falciparum, and here enter strain theory. Can I have the next slide.

It's a very interesting idea and the more I read up about it and participated in this debate, the more I was drawn to the originality and the elegance of the hypothesis. And it postulated that plasmodium falciparum strains exist because of inselection on polymorphic determinants causes pathogen populations to self-organize spontaneously into discreet antigenic types that could be maintained for long periods of time or undergo cyclical or cleoptic [misspelled?] fluctuation.

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Very influenced by a number of [inaduklbe] mathematical models coming from the Sudan with Professor Ron Anderson and his then graduate student Sumatra Day, and in several papers he put forward this key definition of what they mean by strain theory, and it is an elegant idea.

The idea that antigenic variation on the immune selection of polymorphic determinants following an original infection can structure a population such as variants coming in to express the same antigenic variants will not succeed and thus spontaneously, the whole population will throw itself out into strains is an elegant and interesting idea.

The question is, is it true? Can I have the next slide, please?

There are clear assumptions of strain theory and they are questionable. Assumption number one is obviously, the malaria in humans is essentially controlled malaria levels, parasite levels in human populations, the age structuring of the malaria is controlled by strain specific immunity.

Point two, that the amount of strains present in endemic areas are independent, i.e., when you've got two different parasite clones in you they will not affect each other in a sense of an immune response against one will now affect the other either in growth rate or transmissibility as required by the mathematical model which of course, we can't go

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into, but we are pointing at certain assumptions.

The individuals develop lifelong variant specific immunity and since the population is not a single unity on the strain theory, it is a conglomerate of strains; the basic trace reproduction number for each strain must be low. Can I have the next slide, please.

So once again to quote from Dr. Anderson in a seminal publication of theirs, *The Population Structure of Pathogens: They All Have Immune Selection*. It starts to hang on this question of unique PFMP-1 repertoires, "The emergency of discreet, stable antigenic types through immune selection underpins the strain theory of malaria transmission." Dr. Rosen specifically, "that the human immune response against PFMP-1 can cause the population to self-organize into antigenic types characterized by unique PFMP-1 repertoires; however, it remains to be determined by molecular methods whether isolates from individuals with different clinical syndromes are characterized by unique PFMP-1 repertoires."

Ladies and gentlemen, we move on to consider this question. So are strains structured by unique combinations of PFMP-1 genes? Well, PFMP-1 strains are certainly highly variable, as pointed out with considerable clarity by Professor Day, but they are constrained and they clearly fall into distinct subgroups.

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It is our contention that these subclasses essentially occur in all isolates tested and the key relevant reference here is part of Thomas Lavstasvin [misspelled?] in *Malaria Journal* in 2003. Can I have the next slide, please.

What we see here which is difficult to show this sort of slide, but it is that by using the complete genome sequence, i.e., not looking at what we think is a misleadingly high variability picture painted by using small sequences based on a very small end-term region and highly variable end-term region of the molecule, but using sequences of the whole gene available at that time.

Only through the whole genome sequence of clonal 3B7 one can see that *plasmodium falciparum* 60 var genes clearly subgroup essentially into types A, types B, and types C with two transitional groups of A's that look a bit like B's and B's that look a bit like C.

So based on that fundamental piece of data which has never been augmented by further whole genome, whole PFMP-1 sequences and I would emphasize that I think the end-term of those sequences in the DBL alphas never look as though they are related, but once you start to look at the whole gene, you can clearly see they fall into classes.

I refer you to Thomas Lavstasvin's poster in this conference, 316-C the essential observation being the that the

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60 var genes group into essentially three families, A, B, and C. The parasites causing severe malaria in children express in real-time PCR assays limited a more conserved set of PFMP-1 in parasites causing uncomplicated malaria in children.

Thus limited genetic diversity of PFMP-1—and I would emphasize this is where Professor Day and I do differ—we consider that the genetic diversity present in PFMP-1 is constrained and rather more limited than her analysis would suggest and appears to reflect a somewhat limited functional diversity. Next slide, please.

The second observation seems incompatible with unique PFMP-1 repertoires. Parasites causing malaria in young children who have not yet required immunity express semi-conserved PFMP-1's associated with severe disease syndromes the so called, "PFMP-1 severe malaras." Can you go back, please? I refer you to the poster 444-C upstairs at this conference by Pamela Megastrato [misspelled?].

A third molecular observation seems incompatible with unique PFMP-1 repertoires as increased transcription of PFMP-1 var genes groups A, B, but not C are associated with severe malaria in young children. I refer you to poster 444-C, Pamela Stalback.

So to try to bring the argument to some kind of conclusion, again quoting Professor Iala, "where there are

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sectional interbreeding organisms, the individual genotype is a phenoral [misspelled?], what this involves is the gene II and a few individuals and encompass most of the genetic variability of the species."

For instance in humans, another sexual reproducing organism, perhaps 10 to 20 of us would have well over 80 percent of total genetic variability available to human beings. For functional as opposed to DNA sequence or protein sequence variability in PFMP-1, perhaps one individual with it's 60 var genes essentially has all the relevant genetic diversity available.

That's an extreme position. It might be three; it might be five; it's not going to be more than that. Next slide, please.

Okay. And here I have not been able to demolish strain theory. I think it's an elegant theory which is really rather difficult to dispute at this point. There really is not enough data to disprove it, but if this observation is true, I think strain theory is held well below the water line.

Based on a comparison of total var gene diversity in three separate P-falciparum clones, one which is available to everyone, 3D7E and two, again, wild-type isolates, FCL-3 where they currently are being sequenced in the Sanger Center in Cambridge, Thomas Lavstasvin who's has access to these

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databases and has done something of a preliminary analysis which suggests the following striking point.

That intraclone PFMP-1 diversity is actually greater than interclone PFMP-1 diversity. If that is true, strain theory is dead. So once again to summarize, molecular evidence shows that isolates from individuals with different clinical syndromes are unlikely to be characterized by unique PFMP-1 repertoires.

And finally—finalize—we must conclude that if malaria parasites are not structured into strains by unique repertoires of PFMP-1 then with respect to this characteristic at least, all parasites are created equal. [Applause]

MALE SPEAKER 1: We should ask each other; I think, five minutes to prove a point.

KAREN DAY, Ph.D. (New York).: I think the point that I made at the beginning of my talk about looking at the same picture and seeing different things is very valid after you have heard both our presentations.

I just want to ask David really to justify the statement that the intraclone diversity is greater than the between clone diversity. I think what the data that he is referring to, the way that group's var genes is an unfunctional characteristics; whereas if you group them on DNA sequence characteristics, differences in DNA sequence at 96 percent

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homology, you would see that the between clone among clone diversity is extraordinary.

So that's the semantics of the debate where it's at now. Could I ask David to comment on that, please?

DAVID ARNOT, Ph.D. {Edinburgh): Okay. Can we go back to Thomas' paper-picture from my presentation? The sequence alignments—we need to go back to my presentation and can we go to slide number—go further down. It's not that. Further down please, I don't know which number it is, but if you pull them up I will be able to show it. It is number 12. Put up number 12, please.

All right. Well the answer to that is—well I don't think we need the picture—well we probably do if we can get it. I think that the answer to the question clearly is the difference between looking at whole genome sequence and observing that there is clear linkage to sacrolibrium [misspelled?] across the genes that i.e. that fight [inaudible] and indeed upstream regions have always been linked to related three prime downstream regions is much more informative than looking simply at small, sharp regions of what admittedly are extremely variable molecules.

That to my mind gives you an accurate and indisputable picture of the amount of sequence diversity there is in a small region of the end-term of these molecules. It gives new

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insight into the overall relatedness of these families.

Now I would certainly—the hypothesis that these families constitute very similar function aspects of PFMP-1 physiology we know is to be fully established. Nonetheless it is striking and it is not what one would expect whether to be historic diversity of PFMP-1 that has been referred to by numerous authors in the field.

The point is there is a weakness of strain theory that requires that you have very large repertoire, an alarmingly large repertoire, I think, of PFMP-1 genes in the plasmodium falciparum genome.

I would summarize by admitting that one of the things that is clearly deficient in the understanding of the field as a whole is what is the biological function of PFMP-1 switching in the parasite. I think we do not understand enough about this, and many of the analyses are firmly weighted to a sequential, A is on, A goes off, B comes on, C comes on, D comes on. This model is largely based on somewhat unproven assumptions from Japan on mitogenetic variation.

The functional constraints of PFMP-1 which has to bind to ligands are very different from the [inaudible] surface antigen, and indeed, the overall level of polymorphism in the two genes is lower in the malaria situation. I think it's also quite possible to put forward very different hypothesis to

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sequential var gene expression.

It is quite conceivable that upon first infection, the parasite is expressing many PFMP-1's in order to try to assess what the immune and physiological status of the host is, and in that sense, PFMP-1 molecules are not so much immunoevasion switches, but binder sensors for the parasite. We don't know enough about this question. We haven't fully uncovered the secrets of plasmodium falciparum mitogenic variation.

MALE SPEAKER 1: I think it is open to the floor now.

FEMALE SPEAKER: Yes, I believe it is.

MALE SPEAKER 2: [Inaudible]. Go ahead.

MALE SPEAKER 3: I think both Karen and David gave a good overview of the PFMP-1 plasmodium falciparum. [Inaudible] you can think that characterizing parasites out of the field. We know from many studies that there are occupied parasites out there, some that form a vex, some that do a PHP connect. And we also know that parasites some are more antigenic than others when it comes to surface activity which we assume reflect PFMP-1.

Now then sequence is not important? I do believe that sequence is important, and when you look at the activity or interactions with the ligands, it is not only sequence but rather shape. So I would argue that somehow there is occupied or motifs occupied of PFMP-1 sequences in different PFMP-1's

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that if we look carefully and compared the expressed genes in relationship to occupied receptor prevalences or preferences, you would indeed find differences in the PFMP-1 repertoires. Maybe not on the sequence, but maybe at the ligand of an immunoacid [misspelled?] could be similar to each other, maybe rather shape than sequence.

So I would say that the [inaudible] of course, they had to function and develop in order to do something, and I think PFMP-1 is the same. PFMP-1 adhesives, they want to bind somewhere. We know that they form certain adhesives to the repertoires, and therefore, I would say that Darwin would be right, but some interpretations that you made might not be.

KAREN DAY, Ph.D. (New York).: Well, thank you, Matt. I think probably the data that I know best—and I know it's not all the data in the series wrote by Joe Smith—and Joe has been trying to identify adhesion motifs, and what's fascinating is that in different var genes within 3D7, you can't find the identical sequence binding to, say for example, CD-36 or arcan 1, that there are different sequences that are able to do this. So you can't just identify conserved regions of these genes so easily.

We have worked with Gil McVene from Oxford now looking at recombination within these sequences, and the recombination that is going on is extraordinary. It's hard to imagine how

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you maintain function with the level of recombination that we are seeing. And we are looking at, you know, very related parasites taken at the same time within a population and var genes within an isolate and between an isolate; and it's just hard to see this extraordinary sequence diversity. And I think the problem we have got is trying to understand what that extraordinary diversity means, and to me, the most plausible explanation is antigenic diversity, and somehow maintaining conservational function.

These sequences are—if we to draw a tree of all the sequences we've got, we've got a linear tree that would go from here to the ceiling. They just—they are very, very different sequences and the branches are long and that's because of recombination.

MALE SPEAKER 4: I would like to [inaudible] speech about the chemical tree that you have some infection that are pieces of interaction with the receptor repertoire that are relevant to the different receptor interactions. That's how I see it.

MALE SPEAKER 5: Well I am a molecularly challenged, old school parasite immunologist, so I have a really simple-minded question for both of the debaters and it goes like this. If I were infected by a falciparum parasite tomorrow and I were to be denied any medical treatment, should

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I be concerned which parasite infected me—or to put it another way, is there such a thing as a benign falciparum parasite or perhaps a particularly evil falciparum parasite or would I probably get sick no matter which parasite infected me? And I would also like to hear the answer to this question but also the argument why each of you would answer either in the affirmative or the negative.

KAREN DAY, Ph.D. (New York).,: Now David has suggested that I go first, so I will have crack at this. Okay. I think you have to take two models—and I think Ken Marsh has articulated these models previously—is that you either get sick because parasite density gets high, and that could be because you see a parasite bearing variance that you've never seen before—

MALE SPEAKER: But I haven't seen any—

KAREN DAY, Ph.D. (New York).: —but if you haven't seen any variance before we know you will get sick and I think then it probably doesn't matter what the variants are. I think the more important thing is in the African child in an endemic area, a New Guinean child in an endemic area where they have some immunity and what parasite gets through and causes no disease and what parasite gets through and causes disease, and the background of immunity really clearly does influence that.

So my argument would the parasite that is the most

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different to what you have seen before, must be the parasite that makes you sick. Now you can superimpose on that different biological characteristics of the parasite like different adhesion characteristics that may make it fitter cause higher densities make you sick, that could be an additional compounding event. But my view of the world is that the lack of immunity to a parasite is what is going to causes a high density of infection and make you sick, and whether you get really, really sick may depend on some of these other features.

That's the way I view the world, and I think the data that Alyssa Barry, who has really been the prime driver in generating the verging diversity data that we've got, I think that data strongly—if we believe that these sequence variations that we are seeing underlie antigenic differences between the wild-type, you see this so clearly.

These repertoires are very, very different, and so that's being driven by something. It can't—I think it's not just—it is of course a function of recombination, but it's being driven I think by the immune selection argument.

DAVID ARNOT, Ph.D. {Edinburgh): To try to answer Laz's question in the spirit to which it was offered, I think there is malaria therapy data that indicates that they are aware of differences in variants in strains that were kempt to treat patients who had tetraspilus [misspelled?] with malaria by

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giving them a dose of malaria which would cause fevers and kill the sporozoites.

We don't—and I don't—have a new explanation of why they are aware of the differences in variants in these parasites. It could be related to PFMP-1. Dr. Troy and I personally doubt that it is, and I think that long-term passage of the laboratory isolates in the absence of transmission will select for types of parasites that clearly would not survive in the wild; and clearly if our knockouts are clearly viable possibly we could even knockout the whole viral repertoire in viable parasites. I think we can't answer at this point [inaudible].

MALE SPEAKER 3: Well, I will join Laz in his—well as a relatively young member of his old school parasite immunologist, I guess. There is one thing that I haven't grasped. I understand that the interpretation that you are putting on all this molecular work reflects our understanding of pathogenesis related to a sexual stage, right, but what I haven't heard is how all this relates to what is most important for the parasite and that is the generation, survival, and transmission of sexual stages. Can you comment on that?

KAREN DAY, Ph.D. (New York): [Inaudible] my lab a number of years ago showed very clearly that there are antibodies in children living in endemic areas to the [inaudible] infected cell, which indeed, you know, turn out to

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be antibodies to PFMP-1, so there is a direct effect of this immunity on the gametocyte. But I also think there's an indirect effect, by regulating asexual density, you regulate the potential for production of transmission stages.

Now Richard Carter's famous chapter in the Warnsdorf & McGregor book shows very nicely that the production of gametocytes of falciparum is a density-dependent process. You've got to keep a certain asexual density in order to produce gametocyte [inaudible].

So I think for example under the conditions of drug treatment. And so by regulating asexual density, you regulate transmission potential.

DAVID ARNOT, Ph.D. {Edinburgh): I have nothing to add to Professor Day's very good explanation.

MALE SPEAKER 7: I wonder to what degree this debate—like many debates—turns on semantic issues and to whether we are clearing what we mean by strain and strain theory. Just to kind of site, this is coming from a person at Laz's. I mean, my theory would be that for sure I would worry about what parasite I got. David's answer to that is that maybe this is sort of a result of parasite I got out of conditions. I mean it's all very [inaudible] it's to do with their being induced in populations, which two vary—the question is, does a transient collection of genes equal a strain or as one of the

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quotes you gave from IR, it's just a transient phenomena, but it doesn't essentially fit the strain theory because strain theory requires some degree of structuring.

And I think it's needed a definition of what you exactly mean because I came into this debate definitely on the side of strain theory. I moved towards Dave as he went along, and then as I got started to thinking about it, I had gone back to thinking about there is a little bit of strain and a little bit of structure.

So the question I wanted to ask you about is, can you agree on a decisive question because usually things are decided on the facts rather than an opinion. You come to a point where you say, well this is true then—and Dave tried to do that. He put to the—he used some hypothesis statement and said, if this is true, that's [inaudible] he said that's a decisive test, and if not, then I would say, well you don't. Could you give a decisive test; and then I have we will have to ask Dave does to say whether he knows the evidence is there.

If we can agree on a decisive test, we will make it down to the end of the debate. [Applause]

KAREN DAY, Ph.D. (New York).: Okay. I feel like I am sort of in the spotlight here for sure. The—if I could perhaps go to the last point first. I think what we are seeing in reality, if you just accept the sequence diversity definition

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as defining different var genes at this point in time, what we are seeing is a continuum of diversity that—and it's very early days, Kevin. I think the jury is out in some way.

What we have to actually do—and I think the clear question is to define the population genetics of the var genes and I think that means sequence diversity initially and random sampling in a classic population genetics way and getting down and looking at how var genes are organized in populations.

The snapshot we have got at the moment suggests some continuum of diversity, but it also does highlight these common genes. Now I think let's home in on those in terms of the variance issue.

One could ask, is everybody finding certain genes associated with severe disease just simply because they are in every parasite and therefore there is very high probability of them being transcribed in a particular infection because there is a high probability if you look at the denominator.

So I think the question that we need to ask is what is the organization of var genes in populations? And we can see already that there is a vastly different organization in a part of a New Guinean village versus in the African village, and you have to then take it a step further. What is the organization, you know, in a village 300 miles away and in a country thousands of miles away? We've got to work this out because as

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far as I can see the diversity is extraordinary.

I think the typing system that has been presented by David and various colleagues where they have ordered var genes into types is extremely useful, but it is speaking to certain functional characteristics and that's a very useful framework. But I think at the end of the day, the population genetics is going to come out of the sequence variation.

DAVID ARNOT, Ph.D. {Edinburgh): I think once again, we've sensed slightly an aura of Kevin's capacity, and I think yes, I am trying to see if there is a killer experiment and if there is killer data. I think what we want to put up on there is as good as we can come up with.

If intraclonal diversity is high, higher than it is between different parasites, then that says to me the parasite is—each individual parasite—has evolved a fairly stable system which does exactly what it is supposed to do and the variation it needs to do all the things it needs to do is there in one clones—and essentially in all clones. It might be in three or four clones, I mean we could take an extreme position and say that it's on one clone.

I think that if that turns out to be the case then the situation is settled. This is Thomas' work we are talking about, and Thomas and I worked out with these so-called "killer tests" last night over a few beers, so it's not binding

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and a firmly based theory, but it strikes me as the best we can do at present.

I think it will hold as a test. It remains to be seen whether—after a fairly strong spin on it—whether it holds up, but if intraclonal diversity is greater than interclonal diversity, there are no strains because things are simply moving along the way for any form of strain theory.

KAREN DAY, Ph.D. (New York): I think my final comment in response to Kevin's question would be that the strain collapses down to the individual var gene. It's very simple. If what we are seeing is a continuum of diversity, which is what I believe the system looks like at least in Africa then the individual var gene is the strain because it's the difference at that level between parasites that defines whether you will get super infected and whether you will transmit.

DAVID ARNOT, Ph.D. (Edinburgh): I have nothing to add.

MALE SPEAKER 1: Okay. I think we have run out of debate. We can close here. I thank Karen and David and call on the [inaudible] needs to work on this area and come back for the next meeting to answer the question. Thank you.

[Applause]

[END RECORDING]

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