National Institutes of Health
Brain Disorders in the Developing World: Research Across the Lifespan (BRAIN)
Webinar Full-text Transcript

"Iron, Malaria and the Brain"

Presented by Dr. Chandy John
July 25, 2013

Chandy John: My name is Chandy John. I'm a pediatric infectious disease specialist working at the University of Minnesota. My research is in malaria, and I've done research in Kenya and Uganda for the past 16 years. I'm going to be talking about iron, malaria, and the brain today.

It's a particular pleasure to talk about it in the context of the Brain Disorders in the Developing World webinar series, because our research in Uganda on malaria and cognitive impairment in children started 10 years ago as a result a grant from Fogarty through the Brain Disorders in the Developing World RFA. And subsequently, all of our work has been supported by the Brain Disorders in the Developing World program. So this is a direct offspring of that work.

Getting right into what I'm going to be discussing today, I'm going to talk about malaria, iron deficiency and the brain. I'll talk a little bit first about malaria and about iron deficiency as global problems. Then talk about the interactions between malaria and iron deficiency, which are very interesting and make treatment of iron deficiency in malaria-enedmic areas a complex endeavor.

And then finally, I'll talk about the effects of malaria and iron deficiency on the brain itself. That will lead to a discussion of our current and ongoing study (the study is still in process) of iron treatment in severe malaria. And I'm going to present, for the benefit of this brain-interested audience, some very preliminary study results on behavior in these children.

These are not results you can base a conclusion on. They're mostly results to show that the kind of testing we're doing is applicable in this setting, and we'll have to see how the final results play out. And then I'll talk about conclusions we have so far from the study.

So moving on to talk about malaria and iron deficiency-- Chris Murray's article in Lancet estimated that malaria still causes more than a million deaths annually, about 90% in Africa. That makes P. falciparum still the single deadliest organism in children less than five years of age worldwide. And indeed, most deaths from malaria are in children less than five years of age.

Iron deficiency is an equally global and vexing problem. It's estimated that almost four and a half billion people have some degree of iron deficiency, and 800 million people have iron deficiency

severe enough to lead to anemia, including 42 million African children less than five years of age. And iron deficiency can have acute effects, like lethargy, impaired immunity, and if very severe, even mortality. But it also has the chronic effect of cognitive impairment.

So this is an overlay of maps of iron deficiency and malaria. The map shows the prevalence of iron deficiency. Ignore the different colors. All of those areas have iron deficiency. Some of them have deficiencies in other micronutrients as well. You can see that if you look at a map of malaria endemicity-- and again, every non-green-colored area has some degree of malaria transmission -- you can see that there's a great deal of overlap between areas that have iron deficiency and areas that have malaria.

So why are children in low- and middle-income countries vulnerable to iron deficiency? There are several reasons, including that in the first two years of life, you have rapid growth, and growth requires iron. So you're predisposed to becoming iron-deficient, may not get enough on board to have all that you need for rapid growth. In low- and middle-income countries as well, many children have a diet that's low in absorbable iron but also high in phytates.

The example provided here is the cassava, which has only 0.27 milligrams of iron per 100 grams but has almost 624 milligrams of phytates, which inhibit iron absorption. So you're not getting much iron to begin with, and then the iron you do get is not well absorbed. Also in most of these areas, fresh fruits and vegetables are seasonal. So some of the best sources of iron, other than meat, are not available year-round.

But there's another interesting factor that may contribute to iron deficiency, which is malaria. Children with malaria are often iron deficient. And while there may be many factors that cause this, one of them is probably the pro-inflammatory state associated with malaria.

In malaria, you see increased levels of large number of pro-inflammatory cytokines. But you also see increased levels of urine and serum hepcidin. And hepcidin is the major regulator of iron homeostasis. When you increase your hepcidin levels, you inhibit iron transport. It decreases iron transport across the gut, and it tends to trap iron in the liver and reticuloendothelial system so it's not allowed to be released to peripheral red blood cells or to the brain.

Interestingly, though, iron deficiency may protect from malaria. A study by Drs. Gwamaka, Duffy, and group in Tanzania showed quite convincingly that iron deficiency was associated with protection from clinical malaria and death in children who lived in a malaria-endemic areas. A study on the flip-side of things, looking not at iron deficiency but iron supplementation, showed that iron supplementation to iron-replete children can result in persistence of parasitemia and an increase in the risk of subsequent malaria. This is probably because iron is an energy source for the parasite, and also because iron helps to inhibit nitric oxide, which is a major defense against blood and liver-stage parasites. There are probably other reasons, as well, why iron increases the risk of malaria.

So again, since this is a brain-focused group, what effects can malaria and iron deficiency have on the brain? Well, the first R21 study that was supported by the Brain Disorders in the Developing World RFA allowed us to show prospectively and for the first time that about a

quarter of children with cerebral malaria have cognitive impairment two years later. And these deficits were primarily in the areas of attention and working memory. If we look at all the kids who get cerebral malaria and then look at this percentage, it translates to about 200,000 children per year at risk for cognitive impairment associated with cerebral malaria. So a lot of children at risk from cerebral malaria.

There have also been subsequent studies that have shown that repeated uncomplicated malaria seems to have adverse motor effects, and that malaria, iron, and anemia together, lead to problems with motor activities. In 10-14-month-olds, uncomplicated malaria was negatively correlated with motor activity. In slightly older children, malaria affected motor activity indirectly via a decrease in hemoglobin. So both severe malaria and uncomplicated malaria seem to have adverse effects on the brain.

Iron deficiency has been well described as a major cause of developmental impairment. Iron deficiency during brain growth affects myelination. It affects hippocampal and striatal process. And it affects the dopamine neurotransmitter system.

Iron deficiency leads to impairment in a number of areas, including attention, speed of processing, recognition memory, and emotional and behavioral development in particular. So children with iron deficiency often exhibit a particular behavioral phenotype, which is increased worrying and hesitancy, impaired reward-motivated behavior, and less positive affect. These are data from studies that were done by Betsy Lozoff and her group.

Other groups have built on these studies that Betsy and others did, showing very clearly that iron deficiency is associated with developmental impairment. In a Lancet series of articles on child development, the authors conclude that iron deficiency is one of the foremost important factors preventing 200 million children in low- and middle-income countries from meeting their developmental potential. So huge issue, as far as the brain is concerned.

If we look at malaria and iron deficiency may affect anemia and cognitive impairment in a very simplistic model, here's how it would look. In a healthy child, you have normal intake, so a normal amount of iron going into the intestine. And it crosses the intestine appropriately. It gets transported by ferroportin to intracellular areas and goes to the blood and bone marrow, to the liver and reticuloendothelial system, and to the brain. And you have a child that does not have anemia and has normal cognition and behavior.

In iron deficiency due to decreased iron intake, you now have less iron coming into the intestine. Therefore, less iron going to the blood and bone marrow, the liver and reticuloendothelial system, and the brain. This leads to both anemia and impaired cognition and behavior.

In malaria, in a situation where you have normal iron intake, even with normal iron intake, the inflammation associated with malaria increases hepcidin and decreases ferroportin. And so you have less iron transported across the gut, leading to less iron going to all the areas you want it to go to-- and as a result, anemia and impaired cognition and behavior. In addition, you have direct effects of inflammation from malaria and direct effects of malaria leading to bone marrow suppression, hemolysis, and removal of red cells in the reticuloendothelial system, also leading

to anemia and impaired cognition and behavior. So through inflammation, through hemolysis and removal of red cells and bone marrow suppression, and through the effects of inflammation on up-regulation of hepcidin and down-regulation of ferroportin, you have adverse effects on iron distribution in a child, even if the child has normal iron intake, with malaria.

If you combine them together, you essentially get a synergy of adverse effects. The decreased iron intake is now exacerbated by less transport across the gut, which is then exacerbated further by less transport to the areas iron should go because of a decrease in ferroportin. And you have worsening anemia and worse cognition and behavior.

I call this the iron deficiency/malaria axis of evil, where you have iron deficiency and malaria together leading to worse cognitive and neurobehavioral development than either one that would occur in either one of them alone.

I should just note that I just saw that Betsy Lozoff joined the group. Betsy, I just covered some of the reviews of your studies talking about impairment in cognition and development in children with iron deficiency, a couple slides ago.

So this brings the question up of how should we treat iron deficiency in children with malaria? The current WHO recommendations are that if you see a child who is iron-deficient at the time of the malaria episode, you should provide iron to them if indeed they are iron-deficient. And this is in fact what goes on in practice in many African countries. If a child comes in with malaria and they're anemic, when they leave, they get malaria treatment. They're also started on iron treatment.

But this could lead to suboptimal repletion of iron and worse blood and brain outcomes because of the pro-inflammatory state that, as I outlined, leads to poor iron repletion. And it may also lead to an increased risk of malaria recurrence or a lack of clearance. Because parasitemia, even if you've cleared it microscopically, may still be present in the body, and iron is essentially providing food for the parasite.

So this led to our current study that we're doing, which is Acute versus Delayed Iron Treatment in Severe Malaria. This study has been sponsored by NICHD. There's a series of studies on iron deficiency and malaria that was sponsored by NICHD in collaboration the NIH Foundation and the Gates Foundation. Fayrouz has been involved with these studies. And this was in support for the study I'm going to discuss right now.

The picture there that you see is of Sarah Cusick, whose office is right next to mine. She is our nutrition specialist, who's been instrumental in guiding us on considerations of iron deficiency and malaria in this study and has been an important co-investigator in the study.

If I were to summarize this study pictorially in two pictures, and leave you with an image that summarizes what we're trying to understand in the study, is when we give iron to iron-deficient children in malaria-endemic areas, are we feeding the brain or feeding the parasite? And that's an evil Plasmodium parasite coming out of a red cell. Our goal is to feed the brain but not feed the parasite.

The rationale of this study is that malaria-related increases in hepcidin levels are resolved by about four weeks after the episode. So the question is, will iron given four weeks after the malaria episode lead to better iron repletion and more resolution of anemia? Because iron is supplied when parasitemia is cleared, so you see the parasite Xed out there. And better cognitive and behavioral outcomes, where you see the strong brain.

Our study aims were, in children with severe malaria, to determine how acute versus 4-week-delayed iron treatment affects iron repletion and risk of anemia, risk of clinical malaria, and cognitive and behavioral outcomes. This study was built on the severe malaria pathogenesis study is also ongoing and has been supported by NINDS and Fogarty International Center through the Brain Disorders in the Developing World RFA. In this study, we're enrolling children with cerebral malaria, severe malarial anemia, and community children aged from 18 months to 12 years, and looking at parameters of information, endothelial cell activation, and comparing these to neurologic, cognitive, and behavioral assessments at discharge and then 6, 12, and 24 months post enrollment.

The idea of the study is to find out what causes these impairments, because we know they exist. Then once we know what causes them, consider interventions that could decrease cognitive impairment in children with severe malaria. So we nested the iron study into this ongoing study.

In the iron study, we targeted enrollment of 300 children, 100 in each group. All of these children were under five years of age, because that's the peak age for iron deficiency. So it's a smaller age range than the CM studies, 18 months to 5 years. We screen them for iron deficiency using the criteria of zinc protoporphyrin greater than or equal to 80.

There is no perfect screen and in fact, no really great screen for iron deficiency in children who are acutely inflamed, like those who have malaria. Acute inflammation affects almost all markers of iron deficiency. But zinc protoporphyrin can be measured immediately, so you can see a child is iron-deficient when they're not right there on-site versus some other markers. And so this is what we use. And children who had iron deficiency were randomized to receive iron sulfate for three months, either at enrollment-- that was the acute group-- or one month later, and that was the delayed group.

So the study assessments. For iron deficiency, our primary deficiency was the zinc protoporphyrin level. And secondary assessments are using STfR and ferritin, which we measure later in the sample. We're also looking at hepcidin, both acutely and later on. And markers of inflammation like alpha-1 glycoprotein and CRP, which we can both use to see the degree of inflammation and also use to correct for ferritin levels if the CRP's above a certain level.

Our outcomes are iron deficiency, as defined by any of those measures, and anemia at six months. Malaria episodes or hospitalizations at 12 months-- so that's during the 12-month period, not just at 12 months. And then cognitive and behavioral outcomes at 6 and 12 months. And the cognitive effects we're looking at are attention, language, motor, visual and spatial memory, and executive reasoning. And the behavioral effects we're looking at are inattention, fear, and positive affect.

And our study schematic here is that the ongoing study, the CM study that I described early, it enrolls the kids at zero months and then assess them again at 6 months and 12 months. We get a CBC initially and get plasma cytokines and cognitive neurologic testing at zero, 6, and 12 months.

And all of those studies are used in the iron study as well, in the new study-- which I should really refer to as the iron study, because it's not new now. It's been going on for three and a half years. But in that study, we now add to this by looking at iron inflammatory markers during the behavioral assessment. And those who are randomized in the acute arm start iron acutely. Those who are randomized in the late arm start iron a month later. And then we do the same behavioral assessments as well as doing the CBC and iron inflammation studies at 6 months and at 12 months.

The study was approved by the appropriate IRBs and the Uganda National Council for Science and Technology. And we're registered with clinicaltrials.gov, and we do have an independent data monitoring committee.

So just to give some very preliminary baseline data-- but before I do, actually, to talk about the teams that are doing this work. So our Mulago and Makerere team is up top. And I wanted to point out, in particular, this is Paul Bangirana. This is Bob Opoka. I hope you can see the pointer. This is Richard Idro.

Bob is my co-PI on the studies. Paul is the study neuropsychologist. Richard is our study neurologist. And Andrew Ssemata is known by one and all as the Iron Man. He's the iron study coordinator. We have a fantastic team in Uganda doing this study collaboratively with us.

And then for some of the lab measurements, our lab in University of Minnesota-- here we are at the Minnesota State Fair-- is working with us as well. So a number of people, including in particular Sarah Cusick, who I mentioned before, and Greg Park, who's our lab director. So that is the team working on this.

These numbers are slightly old. I sent the slides in early. So we have enrolled more children since I put this together. It's closer to 200 children now enrolled.

But at the time we last compiled data, there were actually 169 children enrolled, 57 with cerebral malaria, 46 with severe malarial anemia, and 66 community controls. And the community controls are children who are enrolled from either the household or the extended neighborhood of a child with cerebral malaria or a child with severe malarial anemia. So it should be those economically, malaria-exposure-wise, and otherwise similar to the children in the disease groups. At the time we put these slides together, we had 123 that had completed 6-month follow-up and 88 that had completed 12-month follow-up.

We've had 10 deaths. Eight were on initial admission. These were all cerebral malaria patients, so this was not a surprise. Unfortunately, cerebral malaria, even with the best chance, a very high mortality rate. And then there were two deaths over the full follow-up period.

We've had eight children lost to follow-up. And two children in the early period of the study that had iron overdose. Neither of them became significantly ill from those iron overdoses. We've since devised these safety boxes and we've had no iron overdoses since.

This is a slide of baseline characteristics to show that in general, the groups are very similar. For whatever reason, there is a greater predominance of males among the children with SMA, or severe malarial anemia, than among the cerebral malaria or community children group. The hemoglobin levels, as one might expect, are lowest in severe malarial anemia, because that's part of the definition.

And the median ZPP levels were very high in all the groups. And you can see that in the CM and SMA groups, the lowest level was still higher than 80. So all children in the CM and SMA groups have been classified as iron-deficient-- about half in the CC group.

So here we are showing the treatment groups and how they were randomized, immediate versus delayed. So in the CM group, we have 58 children and 30 in immediate and 28 in delayed. In the SMA, 22 in the immediate, 23 in the delayed. In the CC group, we have 16 immediate, 14 delayed, and 36 who did not have any treatment because they were not iron-deficient. So their ZPP was less than 80.

The baseline neurobehavioral development-- and this is a picture of Maria Krupina, who did this work. Betsy Lozoff developed some behavior-rating scales and Maria modified these to work with our study. And these behavior-rating scales are designed to look at the areas of inattention/hyperactivity, fearfulness, and presence of positive affect. And they are done by looking at the children while they are in the cognitive testing with their mothers and assessing behavior with their mothers. And these are done through assessment of videos coded by trained coders who do not the clinical status of the child.

So as I mentioned, this is very preliminary data, and it's all data about children at baseline. So these are children who had testing done a week after they were discharged from the hospital. So they are no longer acutely ill, but they are recovering from an illness. And the point was just to look across the groups and see if we could see any differences in groups in particular.

We can't make any conclusions from this because it's baseline data. We're really interested in what happens six months after kids have received iron treatment. But we were just seeing, would the tests differentiate at all between the groups? And you can see that for this testing of negative affect, that the kids with CM and SMA overall had more negative affect. And this was actually statistically significant in the kids with SMA.

In terms of overall adaptation and cooperation, you can see that again, the children with SMA had a lower median adaptation score. And this was significant as compared to the community controls and non-iron-deficient.

I guess I should back up here and say all of these graphs shows kids with cerebral malaria all the way to the left, SMA next over, community controls who are iron-deficient-- that means, had a ZPP of greater than 80-- and then community controls who were not iron-deficient. So the group

to the far right would always be the one you're comparing the other groups to. So on this slide, as compared to the far right, you see the kids with SMA had less cooperative adaptive behavior.

In terms of social responsiveness, again, the children with SMA had the lowest median scores for that. And interestingly, the kids with CM did not differ much from either the iron-deficient or non-iron-deficient community controls.

Then in terms of social initiation, actually, all three of the disease groups, even the community controls who were iron-deficient, had lower scores than the non-iron-deficient community controls. And here you can see these were all statistically significant. Finally, in terms of positive affect, again, the SMA scores were the lowest as compared to the non-iron-deficient children. And for engagement and interest, again, SMA scores were lower than the community control scores.

Sorry. I'm just going to go back to this for a second. So you can see that for a number of the outcomes, children with SMA at baseline, a week after discharge, were doing worse than non-iron-deficient children. And all this says is that we could see a difference in that group, and the difference stood out, and the coders did not know who was in this group.

So it's teasing out that children with SMA have these differences in behavior at a week after discharge. It doesn't say much about iron deficiency or anything else. It could be that children with SMA, even though they get transfused, are still to some degree anemic. Many of them even post transfusion have a degree of anemia, are weaker, and may be exhibiting less positive affect and less engagement and decreased cooperation for these reasons.

So we really need to find out what the differences are at six months. And we're beginning to get that data, but we don't have sufficient numbers yet to say anything much about whether we can tell these differences at six months, and whether there's a difference between those that got immediate treatment versus delayed treatment with iron.

So as I'm talking about six-month outcomes, what I can tell you so far, if we just call the groups A and B, not saying which is which, is that so far, we're not really finding any difference in zinc protoporphyrin levels or hemoglobin levels between one group versus the other. And these values are so close that it doesn't look like even with larger numbers, we'll end up seeing much of a difference there. So we have to wait till the end of the study, with our full cohort enrollment, more than 240 or more children, to see whether we do finally find differences. But we're not seeing any trends in this area.

We're also seeing no differences in outpatient visits. But in terms of hospitalizations, there's a trend towards higher hospitalization frequency in Group A then in Group B. And we will follow this at the time when we have half of our study involved, that we'll be doing an analysis to see whether this trend has actually become significant. But all we can say right now is there's a trend. We looked at it for one interim analysis, and at that point, it was not statistically significant.

And then for cognitive/behavioral, as I said, that data is complex, and we're still compiling that data. And we don't really have numbers, even with final data testing analyzed to say anything definitive about it.

So our plans in 2012 to 2014 were to complete enrollment and follow-up. And we have ideas for future studies, including a study that Sarah Cusick did get funding for at R03 on iron absorption during and post malarial episode, looking at this with stable iron isotopes. So that study has been started in Uganda, enrolled the first two children in that study. And that should provide definitive answers on the level of iron absorption, because we can trace it with these stable isotopes.

Other things we're thinking about are other ways of improving iron absorption. Or are there ways of decreasing inflammation post malaria so we could give children treatment earlier? Because giving children treatment a month later, even if it does turn out to be better for them, is going to be tricky to make as an Africa-wide public health initiative. It's just a difficult thing to do to get people back to a clinic a month later and start them on iron. So we'd probably get a lot of kids that weren't getting iron. And that's not ideal.

We need to find some better way of doing this. And another better way might be if we give iron treatment with malaria prophylaxis during the period of iron treatment, and see if that makes a difference. So those are the studies we're considering doing in the future.

The big picture questions that these kinds of studies and the data that's been generated by many other groups suggest are-- if malaria-related inflammation leads to iron deficiency, will the absence of malaria lead to less iron deficiency? And it is a question that really has not been addressed or answered. We do have some preliminary data from field studies we did in Kenya to suggest that this may actually be the case.

The flip-side of that question is, will less iron deficiency mean children are more susceptible to malaria and other infections? In other words, if malaria goes down and there's less iron deficiency, does that actually make the population more susceptible, given the data that iron deficiency is associated with protection from malaria? And there's the ultimate question of how do you balance iron supplementation with infection risks worldwide?

So our conclusions are that iron deficiency and malaria are both bad for children's brains and their blood. The iron supplementation in areas with malaria may increase risk of malaria and death. And research on safe ways to give iron to children in areas with malaria is urgently needed. Finally, the timing of iron treatment in children with malaria and iron deficiency may be critical to improving their brain and blood outcomes. And this is what we're working very hard to understand and define, so we can come up with recommendations on how it's best to time iron supplementation in children who have malaria.

That's the end of my talk. And looks like I've got plenty of time for questions. I do want to acknowledge before the questions Dr. Robert Opoka, my co-PI in Uganda, and Paul Bangirana, our study neuropsychologist, as well as Andrew Ssemata, who I haven't listed here, but he's the study coordinator. He's done a brilliant job of holding the study together.

Here at the University of Minnesota, I've mentioned Sarah Cusick, who's our nutrition expert here. Maria Kroupina and Michael Georgieff, who've been very important in the behavioral assessment of this study. And Rea Romero, Elsa Shapiro, study neuropsychologists here, and Greg Park, who's the lab director, who's done a lot of the lab testing for this study.

Michael Boivin at Michigan State is one of our neuropsychology collaborators. And then the parent CM study was funded by NINDS and the Fogarty Institute. The cerebral malaria pathogenesis study was funded by them. And my program officers on that study, Deborah Hirtz and Kathy Michels, have been extremely helpful in providing both support for the study and guidance on issues related to the study. So the study could not have been done without their input.

And similarly, for the iron study that I've described, which is nested within the CM studies, Dan Raiten, our program officer, has been a real stalwart at supporting this study and understanding the importance of this kind of work, and really working to get support for these kinds of studies. So want to thank him as well.

I want to leave you with this last pictorial slide again. These are iron pills. And if you have enough iron, you have healthy red cells and healthy brain. But the interloper of malaria can lead to adverse outcomes for both the blood and the brain. So getting iron supplementation right in malaria-endemic areas is a key to having great blood and brain outcomes for these children. And I will stop with that, and I am happy to take any questions.

Joe Perpich: Dr. John, this is Joe Perpich. Are there other comorbid conditions with malaria that play a role as does iron deficiency in this spectrum?

Chandy John: There probably are, yes. Certainly in these areas, another big problem is helminth infections, including hookworm infections. And hookworm is a major cause of anemia. And it causes anemia by sort of leeching blood in the gut. So hookworm could also be a player in both anemia an iron deficiency. That would be one major one.

Another important element is probably schistosomiasis with hematuria and gut blood losses. Schistosomiasis could also lead to anemia. So that's definitely another important comorbid condition.

HIV is a big issue, and the interactions between HIV, malaria, and iron deficiency are really poorly understood. So supplementation in that condition is also something that needs to be much better studied. Because there are interactions between iron deficiency and HIV separate from the malaria component. Then you bring the malaria component in, it gets even more complicated. But how to best supplement kids with HIV with iron is a tough question.

Those are not the only comorbid conditions by any means, but those are three major ones that also probably have a role. I will say that in this area of Uganda-- well, Uganda had a nationwide deworming campaign for a couple years in a row. And these have been very successful. And this area, in the children which we've studied the incidence of stool helminth infections, including hookworm, is very low. So they don't seem to playing a major role in our study. [00:38:14.15]

Joe Perpich: Thank you very much. There's another question here. Can you read it so that we have it for the recording?

Chandy John: Oh, yes. So yes, and I got this by email, so I'm glad you asked it again, because I was just going to come to this. So the question is, do your data support personalizing-- for example, by genetic testing-- the decision of whether or not to prenatally or postnatally supplement with iron?

Our data don't really address that question. And if you have a follow-up on specifics, as to what genetic factors you think might affect whether you should at least postnatally supplement with iron, let me know. As for prenatally, we're not doing any of that work at all, so I don't think I can comment on that. But I'm interested in what factors you think would be important to assess.

Another question, while I'm waiting for the follow-up to that first question, which is a great question, is have you looked at or planned to look at the microbiome? Thank you for that question. We are not looking at the microbiome, but that's not because we don't think it's important. We think it's very important, but we don't have the study resources to do so.

However, in this same group of 10 studies that's looking at iron and malaria across a number of different spectra, there is a group that's specifically looking at iron supplementation and the microbiome. And they're experts in the area of the microbiome. So that work is being undertaken by that group.

Of course, how changes in the microbiome might relate to cognition is yet another question. So eventually, we may collaborate with that group to look at that, but we haven't yet. But I did want to mention that Michael Zimmerman's group is looking at that. It's incredibly important.

Another question from Betsy Lozoff. Congratulations on undertaking this very important study. It's such a challenging issue to tackle. How are you approaching the wide age range of the children in the analysis of behavior and development?

Thanks for that question, which is really timely, because we've started the analysis of some of the six-month data. And obviously, behavior and development changes in these children with age. So what we're trying to do is use some regression models to see how it changes with age.

We do have control children that don't have iron deficiency, and so we're sort of using them as comparative children. What we're hoping working with our statistician, to do is use these regression models to come up with norms data that we can then compare the disease with, age norms data that we can then compare the disease group to.

But I would love any input you have on that, Dr. Lozoff. So perhaps we can talk or email after this conversation is over. Because it's a tough thing-- age really makes such a difference. And if you have better ideas on how we could do this, I'd certainly appreciate them.

Question or comment from Kathy Michels and Farah Bader. Thanks for a great presentation. We look forward to hearing the full archive presentation. Thanks, both, for coming. I praised you lots, because your support has been the key to getting this work done. Great.

And Betsy said she'd be pleased to think later on this, so that is great. Really appreciate that input. Any other questions or comments?

Joe Perpich: We just have a follow-up to the genetics. The Gates Foundation is really putting a lot of money into the genetics of the malarial parasite in order to find where in its development interventions can be made. And you've mentioned that some of the work in this area that you're presenting was funded by the Gates. Was any of this work at all, at any point related to those genetics projects?

Chandy John: Of the ten grants, there weren't any that were specifically on parasite genetics or human genetics. But there is a study by Carla Hand at University of North Carolina, looking at characteristics of parasite invasion of red blood cells in cells from iron-deficient versus non-iron-deficient individuals. And so how might iron deficiency protect from malaria?

And those studies are absolutely fascinating. Hopefully she'll publish some of the initial results soon. But there does seem to be a difference, and particularly, individuals with microcytosis and iron-deficiency anemia seem to have a resistance to parasite invasion and growth in their red cells. And Carla's now trying to look at why. So hopefully we'll have some of those answers. Nothing specifically on parasite genetics, though.

Joe Perpich: You mentioned-- just wanted to follow up-- you mention four and a half-- what did you say? How many billion iron deficiency? Is this something that the World Health has a major health initiative on as it does for many of these other areas? It's like the equivalence of several vaccine programs the WHO is sort of correcting iron deficiency around the world.

Chandy John: Yeah. I think WHO has a number of guidance documents, and they are the ones that have sort of put iron deficiency at the forefront as a major world problem. So they have guidance documents. They were in the lead on getting pregnant women supplemented with iron, on highlighting iron deficiency as a problem in children under five.

So yes, they are very involved in considerations of iron deficiency around the world, and particularly in guiding policy on how to supplement iron. So this whole iron deficiency-malaria interaction has sort of been a thorn in their side, frankly, because what we'd really like to do is provide iron to these kids in Africa. They need iron for growth and for brain growth. But we have to figure out the best way to do it without increasing their risk for malaria. So definitely high on the WHO agenda, and I'm sure Betsy could say more than that about that than I could.

I got a note from Kathy Michels about if the research capacities that have been developed in Uganda as a result of the program will continue beyond the program. So one of the really great things about this particular study and its support is that there's an emphasis on capacity building. Through this study, one of the people that I highlighted, Paul Bangirana, was able to get his

Ph.D., a joint Ph.D. from Makerere and the Karolinska Institute. And it was the first Ph.D. in pediatric neuropsychology in Uganda, so he is the first pediatric neuropsychologist in Uganda.

Again, through having these grants in place, we got a training grant from Fogarty and NINDS to support research on infection in neurodevelopment. So we have Ph.D. students in epidemiology, immunology, neuropsychology, masters of medicine students studying in internal medicine and pediatrics, masters students in immunology, and students in data management and statistics as well. So a lot of training going on as a result of these studies.

And people involved with the study, including Bob Opoka, Richard Idro, and Paul Bangirana, have all obtained their own grants to study-- in some cases, several grants-- to study issues related to malaria and/or brain problems. Like Richard's doing a major study on epilepsy and studies on nodding disease. And these studies will continue with or without our support. So the studies that we're doing interact with and interweave with these studies. But if the University of Minnesota group left tomorrow, the other studies would go on.

So there's been fantastic infrastructure capacity and development opportunities from this grant. And it's really one of the most satisfying things about doing these studies. As I mentioned, I have the privilege of working with really, really remarkable people in Uganda, and seeing them progress incredibly in research efforts of their own, and developing infrastructure capacity there.

We've been able to get internet access there. We're developing a mentorship program. All of this was started through these programs. So the infrastructure capacity was a major, major part of the Fogarty and iron grants, and now the D43.

Fayrouz clarified for us that Dr. Hand's model suggests that iron deficiency is protective. And if iron supplementation is given, erythropoiesis begins with a subset of younger RBCs produced-- their phenotype has not yet been characterized-- with an increase in susceptibility to malaria, of which the magnitude remains unknown. And they then revert back to normal as they become iron-replete. So that's a clarification of some of the really interesting research that Dr. Carla Hand has done.

A comment from Betsy Lozoff. Possible adverse effects of universal supplementation are a major concern for implementing iron deficiency reduction programs. This is exactly right, and it's one of the reasons why addressing this concern is urgent. Because these kids do need iron. Nobody disputes that. But they're not getting it because of these concerns. I think that highlights the importance of this kind of research.

It looks like those are all the questions and comments. So Joe, I don't know if you wish to say anything else or whether we should close the session?

Joe Perpich: Thank you very much, Dr. John. I keep reading about advances in vaccine development for malaria led by the Gates Foundation. Until I'd seen your PowerPoint and heard your presentation, I really didn't make the connection between iron deficiency and malaria. Your webinar has opened up a window on this research area and its relationship to the work of the

World Health Organization in providing iron supplementation for diets in the developing world where malaria is extant.

So on behalf of all of us, I want to thank you very much. It's been a great overview, and your answers were really very illuminating.

Chandy John: Great, thank you so much. Thanks to everyone for participating and for the great questions and comments people had. This is a great forum to talk on, and thank you very much.

Joe Perpich: Thanks a lot. And thanks again to Fogarty, which has organized this webinar series with additional support from NIDA.

Chandy John: Thanks.