Careema Yusuf: No problem, and I just want to go over some funding announcements just to say that this webinar is supported by a cooperative agreement through CDC.

Tim Davis: Perfect.

Careema Yusuf: And none of the contents are the responsibility of the authors, they are the responsibility of the authors and do not necessarily represent official views of CDC or the Department of Health and Human Services.

Tim Davis: Okay.

Careema Yusuf: There you go Tim, please take it away.

Tim Davis: Hello from the great state of Washington.

On behalf of the APHL Hemoglobinopathy Work Group, I would like to welcome you to part one of a two part webinar series on Alpha Thalassemia. My name is Tim Davis, Chair of the Hemoglobinopathy Work Group and Lead Microbiologist at that Washington State Newborn Screening Program.

Our work group was convened in 2013 through the National Center on Birth Defects and Developmental Disabilities, Division of Blood Disorders, the CDC, and APHL to address issues around hemoglobinopathy testing in Newborn Screening.

Whether your program uses isoelectric focusing, HPLC, or a combination of both for hemoglobin screening, we're all detecting alpha thalassemia through hemoglobin barts. The question is, how do we report it, should we report it, and ultimately, how does this information help the newborns and families we serve.

Through these webinars we will explore globin chain expression and imbalance, the types of Alpha Thalassemias, and how reporting helps with diagnosis and treatment. We'll also look at examples of isoelectric focusing, hemoglobin Constant Spring, HPLC percentages and cutoffs, testing algorithms, and the current status of Alpha Thalassemia Screening across the United States.

Today's webinar focuses on the clinical aspects of Alpha Thalassemia. Our first speaker is Doctor Maria del Pilar Aguinaga. Doctor Aguinaga is the Co-Director and Laboratory Director of the Meharry Sickle Cell Center. She holds an adjunct faculty appointment at the Department of Medicine, Division of Hematology/Oncology at Vanderbilt University. Her research interests are in Sickle Cell Disease and women's health, and her clinical interests lie in the diagnosis of hemoglobin disorders for which she is consulted nationally, and
internationally. She also directs the State of Tennessee newborn screening Hemoglobinopathy Confirmatory and Reference Laboratory housed at the Meharry Sickle Cell Center.

We’ve also reserved time at the end of this presentation for questions.

Welcome Dr. Aguinaga.

Careema Yusuf: Please press star seven to unmute yourself.

Dr. Aguinaga: Thank you Tim. Greetings to everyone. I would like to start by defining Hemoglobinopathies and Thalassemias.

Press the slide please.

Hemoglobinopathies are most common due to mutations in the globin gene, specifically in the coding regions that are going to change the normal amino acid sequence of the globin chain. This produces hemoglobin variants. In the case of the Thalassemias we have deletional types and non-deletional types that are going to lead to a decreased availability of the particular globin chain that is being affected. Therefore, there will be an imbalance in the normal coordinated synthesis of the globin chains of units that we see.

These disorders originated in the tropics. However, due to worldwide migration, they can be found anywhere.

Next the slide.

It is estimated that about 5% of the world population are hemoglobin variant carriers. On this slide, we have the globin gene clusters. We have the alpha globin gene cluster, on chromosome 16 and the beta globin gene cluster on chromosome 11.

The way these genes lie on the genome are also the same way they are expressed during ontogeny.

Next the slide please.

Alpha Thalassemia mutations are probably the most common mutations in the world. Most of the Alpha Thalassemia mutations are due to deletions in the alpha globin gene. But we also have less common, non-deletional types, alpha-thal mutations leading to some hemoglobin variants, that are going to give an Alpha Thalassemia type.

There are at least 50 known deletions, which involve both of the alpha globin genes. They affect the chromosomes, and over 69 known types of non-
deletional types of Alpha Thalassemia mutations, but these are, in general, very rare.

The two most common alpha globin gene deletions are the 3.7 rightward and the 4.2 kilobyte pair leftward. And we can see this right here on the next slide.

In general, in the United States and the southern states, we have one out of 15,000 births affected with Alpha Thalassemia.

In African Americans we have one out of 30 births that will carry an alpha-thal mutation. And one out of 20 births that will carry are Southeast Asian mutations.

Next the slide please.

Most of these mutations are prevalent in people from Southeast Asia. On this figure we have the deletional types, we have seven out of the eight most common, alpha-thal deletional mutations. We can see the 3.7 and the 4.2, the Southeast Asian, the Mediterranean and others.

Next the slide please.

This table, we have the clinical forms of Alpha Thalassemia. When we have four functional alpha globin genes, we have the normal phenotype. Our red blood cell indices are normal, and we have no hemoglobin abnormalities. When we have one alpha globin gene deleted, this condition is called, silent carrier or alpha-thal two heterozygote. Basically, there are no clinical symptoms and the red blood cell indices may be slightly affected. We will see at birth a small amount of bad hemoglobin, one to 3%, which will disappear as the baby grows up and develops.

When we have two alpha globin genes deleted we can have two in the same chromosome, or one in each chromosome. In this case, this called alpha-thal two homozygote, or alpha-thal trait. We have mild anemia, microcytosis, hemoglobin is 11 to 13 grams, MCV 65 to 75 femtoliters, MCH 24 to 27 pictograms. At birth,, we will have little bit more Hemoglobin Barts, three to 6%, which will disappear as the baby grows up.

In the case of the alpha-thal one heterozygote, which is close to alpha-thal trait we have similar clinical features and same red blood cell indices.

When we have three alpha globin genes deleted, this condition is called, alpha-thal intermediate or hemoglobin H disease. Here we have a variety of clinical features. It could go from moderate to severe anemia. Hemoglobin seven to 11 grams, MCV 55 to 65 femtoliters, MCH 20 to 27 pictograms, and newborn Barts, at birth, is going to vary from five to 30%, even though in my experience, when I see 20 to 30% I strongly suspect Hemoglobin H disease.
Hemoglobin H after one year of age is going to vary, as low as 3%, as high as 20%.

Then when we have the four alpha globin genes deleted, this condition is called Barts hydrops fetalis or Homozygous Alpha Thalassemia. The clinical features are usually fetal death with hydrops fetalis, this condition is incompatible with life. Hemoglobin is less than seven, the cells are bigger 100 to 110 femtoliters, and MCH is less 22.9 pictograms. Hemoglobin Barts is usually 99, 100%.

Next the slide.

As we have deletional types of Alpha Thalassemia we also have non-deletional types of Alpha Thalassemia, usually involving hemoglobin variants. For example, Hemoglobin Constant Spring, which has a termination codon mutation, therefore it produces an extended alpha globin chain. Or Hemoglobin Pakse or, other highly unstable hemoglobins, like Hemoglobin Agrinio, Suan-Dok, Quong Sze, or Pak Nam Po.

Next slide.

In Alpha Thalassemia we will have a decreased availability of alpha forming chains, which is going to lead to accumulation of beta chains and gamma chains. The beta fours will form hemoglobin H and the gamma chains will form hemoglobin Barts. These also reduce amount of normal adult hemoglobin, hemoglobin H. Now hemoglobin H is going to produce the red blood cells to be more sensitive to strong oxidant compounds resulting in hemolysis, this can be worsened by oxidant drugs, moth balls or infections. And in general the red blood cells are gonna be hypochromic and microcytic.

Next slide please.

Hemoglobin H disease, you can have hemoglobin H disease with three alpha globin gene deletions or two alpha globin deletions plus a hemoglobin variant like [00:10:02] Hemoglobin Constant Spring.

In general, we will see hemolysis and ineffective [00:10:10]. The co-inheritance with other globin gene defects affect the hemoglobin severity and it is believed and it is seen in hemoglobin H, hemoglobin Constant Spring more severe. And about 50% of these cases usually are due to hemoglobin [inaudible 00:10:25] hemoglobin H plus hemoglobin [inaudible 00:10:28] also an unstable [inaudible 00:10:29]

Next slide please.

How do we diagnosis hemoglobin H disease? First, we have amount of Bart's at birth, the presence of hemoglobin H itself, hemoglobin A2 is generally low but you know in the newborn hemoglobin A2 is low anyway. Total low hemoglobin,
low MCV, low MCH some hemoglobin Constant Spring or their variants in general most patients are from South East Asian origin

Next slide please.

Clinical symptoms, moderate anemia and microcytosis also jaundice, hepatosplenomegaly and some infants may have poor growth and development and sometimes you'll see facial and skeletal abnormalities as well as gallstones, leg ulcers, iron overload due to the ineffective [inaudible 00:11:18].

Next slide please.

What is the definitive diagnosis. You can diagnose at the newborn stage Hemoglobin HPC usually by high performance liquid chromatography where you can determine the amount of Bart's hemoglobin and also the presence of Hemoglobin H. More than 25% Hemoglobin Barts is suggestive of HPC at birth. Then you need to have a confirmatory test, which more now involved other more sophisticated [inaudible 00:11:50] and even DNA analysis. These babies need to be referred to a pediatrician hematologist for diagnosis. If the baby has been transfused before the newborn screening sample was taken then please repeat the newborn screening sample after 90 ninety post transfusion.

Next slide please.

Other recommended tests are Isoelectric focusing electrophoresis, hemoglobin electrophoresis. If you have a baby with hemoglobin HPC please tell the siblings as well and provide counseling for the parents.

Next slide.

In general the alpha thal trait will show no specific characteristics on electrophoresis HPLC or even capillary electrophoresis except for a marginal reduction in Hemoglobin A2 expression. This reduction is more prominent when you have hemoglobin HBC or when the genes are dysfunctional or the Alpha globin gene. HPLC and Capillary Electrophoresis can detect alpha thal in newborns because of the different levels of hemoglobin Barts and the presence of hemoglobin H.

Next slide.

Here we have a three month old infant and this is an IF Electrophoresis and you can see where the arrow is been pointing you can see some Fast migrating bands. You can three. The very first one is hemoglobin H, the next one is Bart’s and the others and you can see others but they are very faint they are multimers of beta and gamma globin chain.

Next slide please.
This is a three year old South East Asian infant and here you can see on the HBL [inaudible 00:13:32] primal HPLC. You can see Bart's hemoglobin, very [inaudible 00:13:37] then you can see some [inaudible 00:13:39] and hemoglobin H above 7%.

Next slide please.

This is an adult a 23 years old adult from South East Asia that the sample was analyzed [Mihari 00:13:56] and we can see hemoglobin A and a very [inaudible 00:14:03] resolution at the end of the [inaudible 00:14:04] among the left, which have the [Biorab 00:14:06] variant 2 chromatogram and right would have the primal total 2 chromatogram. If you see on the right two arrows, the first arrow is pinpointing to Bart's hemoglobin and the second arrow pinpointing to hemoglobin H which point you to hemoglobin A1. So we decided to send this for DNA analysis.

Next slide please.

And DNA analysis revealed hemoglobin H plus hemoglobin Constant Spring and please note the S and the stop should be after the C. It's Constant Spring CS. And DNA analysis revealed alpha Constant Spring alpha is flush South East Asian deletion. So this patient has hemoglobin H plus hemoglobin Constant Spring disease.

Next slide please.

This is a case of Bart's Hydrops Fetalis. This baby who has ... seen last year it was born with 99% hemoglobin Bart's.

Next slide please.

So everybody knows this. It's important because of the wide spectrum in clinical manifestations and severity. You have to regularly monitor hemoglobin levels, monitor the patient's anemia as well as growth and development and assess chronic hemolysis complications, assess possibility of the infection and overall health care monitoring and proper immunization.

Next slide please.

Do not rely alarm the parents if you have done a confirmatory testing for hemoglobin NPC. Provide education of parents to understand importance of following up with their PCP for additional testing and referral to pediatric hematologist.

Next slide please.
Regular visits to the pediatric hematologist and parents should really understand hemoglobin H disease that is a chronic disease and it will require monitoring of hemoglobin levels, growth, development. Provide a list of support services such as local health department, and early intervention service providers. Genetic counseling and education for the parents and the family are strongly recommended and for more information please look at newssteps.org. Thank you very much.

Tim Davis: Thank you Doctor Aguinaga.

Our second speaker is Doctor Michael Bender. Doctor Bender has a broad background in, and a long term commitment to both basic science and clinical medicine, with them dovetailing in the study of globin genes and disorders there of. Integrated with this basic science work, has been a long term commitment to the care and education of patients with hemoglobinopathies. He has run a program for children with hemoglobinopathies in Washington state for over 15 years, and has had funding from the Health Resources and Services Administration and the Washington State Department of Health to develop a collaborative to improve clinical care and provide community outreach for families affected by Sickle Cell.

He provides consultation to the Washington State Newborn Screening Program on hemoglobinopathies and is a Northwest resource for community physicians.

Welcome Doctor Bender.

Dr. M Bender: Thank you so much for this opportunity, Tim. Thank you so much Corrine, for your patience in helping me get set up for this talk.

To start off I want to compare the issues of Newborn Screening for Alpha Thalassemia with that of Sickle Cell disease and some of the metabolic disease. Unlike sickle cell and the metabolic diseases, in alpha-thal it is not about preventing a dramatic acute phenotype, like brain damage and death. In sickle cell disease, it's important to keep in mind, that Newborn Screening reduced the morbidity and mortality of sepsis even without prophylactic penicillin. The key in this case, was that early diagnosis and education of providers and families on detecting fever and how to respond greatly improved outcomes. Plus it allowed genetic counseling leading to more informed decision making.

In Alpha Thalassemia, while not as dramatic, it's really about avoiding a family odyssey of children not thriving, recurrent medical testing, misdiagnoses, treating the wrong condition with contraindicated or potentially dangerous interventions, avoiding tension with the medical providers, and minimizing long term complications, and I'll go into all that. There's much less impact but potentially reach far more children and families.
How do we do this? First, by tapping into the current infrastructure diagnostics. It's about family education and safety teaching, and provider awareness and education. In part because there is a huge educational void in terms of diagnostics and management here. Along with this is the side benefits of allowing genetic counseling and avoiding hydropic pregnancies.

My approach today is to start by reviewing the last major review of looking at hemoglobin H as a core target for Newborn Screening. This was done in 2010 and this is the front page of the report. It is available on the link below. Then to discuss other benefits of Newborn Screening part is in terms of its effect in terms of Alpha Thalassemia trait and then genetic counseling.

When it was reviewed in 2010 they, obviously, decided not to do universal testing. This is from their summary in terms of what some of their concerns were. As it states, the committee felt that they had not heard compelling evidence to suggest hemoglobin H belongs on the screening panel. That there's no compelling clinical need presented. Therefore, more evidence was needed.

So, what were they looking for? One was what proportion of children would benefit from condition-specific treatment. Where do we stand? Well there's some more data but not a lot. They asked what the variation of prevalence across the United States is, and there's more data for that. They asked does early identification improve the health of identified children? And I will go over this, there's some data for that. They want to know what the target is, I'm not going to go over that. Then they wanted to talk about infrastructure and what the expectations are for newborn screening labs and Tim Davis will cover some that and I'll cover some of that as well.

In the report they really wanted to address multiple key questions about bringing testing to hemoglobin H disease. About the condition, screening tests, diagnostics, treatment, economics, and other. In part two of the series, Tim Davis is going to cover diagnostic tests and screening tests.

I'm really going to focus on the key questions about the condition, the treatment, and the economics. I will come back to these systematically later so don't worry about reading these now.

The first talk was a beautiful review of alpha-thal, I am going to do a very focused repeat of some of that. Alpha Thalassemia, it's really about chain imbalance, not about decreased expression. It's what would the unchained, unpaired hemoglobin chains do. In terms of screening, hemoglobin H, it's made of a tetromere of the adult hemoglobin, this is too unstable to be seen on gels and so it is not useful for adult testing. In contrast, in the newborn period, hemoglobin Barts, a tetromere of the gamma chain, is unstable but it's still stable enough to be seen on gels. It provides and easy test to newborns. Thus, providing a window of opportunity, in the newborn period, for an easy, cheap, diagnostic test.
Unlike beta thalassemia, usually the whole gene is deleted. It's quantitative, that the level of hemoglobin Barts correlates with the number of genes deleted.

In contrast, hemoglobin Constant Spring, as mentioned, is not as common as the deletion form of hemoglobin H, but is the most common form of it. It is much more severe but much less called out. It's due to a point mutation that was brought up and it's quite unstable. Usually it's seen on gels.

Why is hemoglobin H Constant Spring so much worse than hemoglobin H? This is a map of the alpha gene low side. There's the embryonic gene and two adult alpha genes, and upstream of the genes is a super enhancer that activates the genes. The two genes compete for the activity of this enhancer. Alpha two is closer, it competes better so you get more expression than alpha one. Normally in humans alpha two is expressed more highly.

What happens when you delete alpha two? There's no competition with alpha two, so alpha one gets all the attention. So there's an up regulation, and you get more alpha one expression than in normals. So that's key in alpha gene deletions, you delete one gene, the expression of the other goes up.

In hemoglobin H what happens, if it's a deletional form, you get up regulation and you get more alpha one than you would normally get.

What happens in hemoglobin Constant Spring? Why is it worse or more severe? Here you're not deleting Constant Spring, it's still present, so you still compete with that enhancer, regulatory region. So you do not get up regulation of alpha one. So you end up making less alpha chains than in other forms of hemoglobin H.

As a result, the gene's presence, you don't make the useful protein, you get competition, you don't get the up regulation.

So what are the concerns in hemoglobin H? Again this was reviewed before but I will go over it again in brief. Some of it is about decreased growth. I'll show some data about height and weight, and bone mass. Ineffective erythropoiesis, which can lead to enlarged spleen and iron overload. But the most common thing is the chronic anemia and occasionally the anemia drops, and gets even more severe.

Potentially this requires transfusion, but most tolerate the anemia on a daily basis just fine. What are the risks for dropping from this baseline anemia and potentially needing a transfusion? Often viral or other infections, can suppress making red blood cells so you can drop. Inflammation of oxidative damage, which can be from some foods, some medications, or concomitant G6PD deficiency.
One of the problems with hemoglobin H is the lack of awareness of the chronic anemia and its etiology, and what kinds of evaluations are needed.

As in beta-thal you can get some iron overload secondary to the ineffective erythropoiesis even if you don't get any transfusions. It's progressive, and when people are older they may need chelation. It's slower than beta thalassemia but it still occurs. If you have hemoglobin H with Constant Spring or other non-deletion forms all these things are just more serious.

As I mentioned, there's knowledge gaps. This is from the summary, last time in 2010, when they looked at how best to treat hemoglobin H, and as you can see there is a paucity of quality literature out there.

What's the natural history of hemoglobin H if you diagnose it early? Kids get started on folic acid to help make red cells. They get monitored with CBC's looking at reticulocytes. Watching iron status, looking at accumulation. That's the main lab and medicine part. The biggest part, is family education. It's awareness that the child has anemia and how to manage it. How to avoid oxidant exposures, like Sulfa drugs, fava beans, moth balls. How to monitor it, which is mostly looking for the signs and symptoms of worsening anemia. Such as being pale, having fatigue, change in their color, decreased perfusion, capillary refill, less energy, just being more fussy. It's about families learning when to be seen and having blood work done, because if they notice these things it's important to have blood work done to see if you're more anemic than usual. It's about avoiding iron unless you are proven to be deficient, not to give it just because you have anemia.

In terms of Provider Education, it is the same as for families. Many general practitioners, family medicine pediatricians, primary care givers don't know much about hemoglobin H. So it's critical providers know about how to diagnose and care for hemoglobin H. It's common, as I'll get into, to give people with anemia, inappropriately give them iron or to transfuse. It's important to teach them that this isn't a generic microcytic anemia, that iron overload occurs even without giving iron, and that iron's contraindicated unless it's documented.

So this is a beautiful article from several groups including, mostly the group in Oakland, from 2011.

It's comparing regular deletional hemoglobin H with hemoglobin H with Constant Spring. Through this the open triangles are hemoglobin H, and the closed are hemoglobin H Constant Spring. If you look at growth the top panel is weight, you see hemoglobin H Constant Spring is much more severe than hemoglobin H, the deletional form. If you look at height, the same thing. The orange line shows changes by age five because for Newborn Screening you really care about things that affect children under five years of age. So really there's a dramatic difference in height and weight for those with hemoglobin H Constant Spring below age five. The top panel on the right is the hemoglobin with is [inaudible 00:30:06] lower and bottom two panels just show that
hemoglobin H Constant Spring you more reticular sites, which means you're working much harder to make blood and your bilirubin is higher showing that you're [inaudible 00:30:20] you're breaking down red blood cells more readily.

This is the big one. This is those children that were transfused and if you look at hemoglobin H the deletional form, very few get transfused before age five. In contrast, by age five about 40% hemoglobin H Constant Spring have required transfusion. So pretty dramatic. So overall about 5% of all forms of hemoglobin H needed transfusion before five years of age. So one of the big questions is how do you know someone needs a transfusion, how does this come about? Well if you know your hemoglobin H and people are monitoring for anemia it's easy to tell. If you don't do that, they often present with a profound potentially life threatening anemia.

So if you're diagnosed with hemoglobin H and you're not diagnosed with newborn screening, what happens? Well there's not any good data for how these children present because most of them haven't been diagnosed or many of them haven't. My own experience and many hematologist experience is a referral for an anemia not responsive to iron. Now this brings us to general pediatrics. If you look at the American Association of Pediatrics, for all newborns, all infants they recommend a hemoglobin screening at 12 months of age. The main reason they're doing this is to detect iron deficiency and iron deficiency anemia, which are very important because iron deficiency in infants can lead to neural developmental problems and have a neural developmental impact so you want to pick it up early. So every 12 month old regardless of hemoglobin status should be tested at 12 months. Unfortunately this is a real mess.

First of all, it's usually screened for cause reasons by screening hemoglobin when then sensitivity and specificity aren't great, but it's also a complex screening algorithm the AAP recommends where they also want to take into account the history for the social economic status, heritage of the family and diet history. So it's a lot of work. Depending of the results they recommend either treating with iron or testing and treating so again complexity and then followup to show correction but one of the big problems, which I'll show next is that this is often not followed and correction is not documented.

That is a big discordance between what's recommended and what's done. So this was a large study of the general pediatric population, which is almost 5000 children who were screened at about 12 months of age for iron deficiency anemia. And depending on where there is a wide range that screened positive or depending on how you screen between 1.5 and 14.5%. That's a large number of infants are involved here. The key things are of those that were found to be anemic less than 25% of those underwent a repeat, so there is ... within six months so there is not follow up in general in study. Moreover, only about 11-12% were shown to have it documented corrected. So a large number of infants are impacted with infrequent follow up testing. It's rare to show documentation.
And often people get iron and there's no follow up. So how many of these kids have to go with age without a trace. We don't know. This is where I feel the odyssey begins that infants are diagnosed as having anemia. But then what happens. So how do you quant- take the importance of what's going on here. I think there's several ways to look at it. So what is this scenario with an infant ... well an infant or child or adult comes to medical attention and then you get put on iron for iron deficiency. Even though they don't need it. They're anemic because they got hemoglobin H not because of iron deficiency. If we start talking about it, if you start asking people most people have stories, they know of people that got put on iron and they keep getting more iron and intermittently they're on iron all their life. Why is this?

Do they have a hemoglobinopathy? Are they truly iron deficient? People don't know. So what happens in real life? Infants don't respond to iron therapy if they've got hemoglobin H. They've got anemia because they got a hemoglobinopathy so they're given iron again often there's a followup CDC but not other diagnostics. Providers often get frustrated with the parents often to get accusations that they're not complying and not giving the iron, they're not given it appropriately. But meanwhile if they've got hemoglobin H, they're getting iron inappropriately. Often there is no follow up and children just float out there. Another scenario if someone develops a profound anemia. In this case they can be transfuse but if it's hemoglobin H often they have a low baseline anemia all the time and they are fine with it. And so sometimes they are inappropriate transfused or sometimes because families aren't educated when they come in they're showing up with this profound anemia much later than ideal when kids are dangerously ill. Another scenario is someone's diagnosis chronic anemia, they don't really evaluate why and some kids end up on chronic transfusions, which is inappropriate.

Less common because of [inaudible 00:37:08] and anemia some children have their spleens removed empirically, which is not necessary here. And again there's often tensions with health care in terms of how people are following up and following guidelines. What really happens in terms of how often any of these scenarios occur it's not known. It just isn't a study. So what is the problems of hemoglobin H is diagnosed late in life as opposed to in a newborn. Well there is delays, potential delays in seeking care and the [inaudible 00:37:45] of living with chronic anemia potentially presenting with life threatening anemia. The growth the weight and height and weight I showed, sometimes chronic fatigue and decreased energy is the odyssey of misdiagnosis and at times inappropriate treatment is common to have multiple evaluations, appointments, labs, recurrent courses of iron, that may be worsening iron overload, which aren't always necessary if someone has hemoglobin H a lot of that isn't needed.

Can lead to unnecessary splenectomy, conflict with providers, there's inappropriate transfusions whether once in a while or chronic and those transfusions are times stress, the financial cost and potential risk of transfusion. And in addition you're not doing anything about the potential genetic risk of a
child with parents of having other children with hemoglobin H or hydropic pregnancies. So going back to the key questions that the panel last time. What's the prevalence of deletional non-deletional hemoglobin H disease? Well it's tough because it's not routinely screened for or tracked in the United States. So there is a lack of data. It's more tough, it's even more difficult to report out hemoglobin H Constant Spring because it's not always on gels. As far as I know only California and Washington State screen for it.

So because there's not great national data, I'm just going to go over the Washington State data, which I've [inaudible 00:39:31] from Tim Davis and then also talks about some of the California data. So this is Washington State data for a 10 year period and first if you look at hemoglobin H, it just goes over the number of cases per year. The big thing is it works out to about 6.3 cases per 100 000 infants screened. In contrast, hemoglobin H Constant Spring, which is more severe is a three year period where there wasn't screening but it occurs at about 10% as frequently as hemoglobin H.

In contrast, I'll come back to this later alpha-thal trait is far more common about 40, 50 times more common. If you look at who's at risk as was brought up in the last talk it's mostly in people of Asian descent but with changes in social habits there's a lot more ethnic mixing. So as a result for the year pointed out here while there's four I guess self-identified as Asian, there's half that amount as other or unknown. So increasingly there's people of mixed race with it. In discussion with Dr. [Ashlow 00:40:53] who runs a thalassemia program in Oakland, he shared that the California experience is quite similar in terms of the numbers and percentages it's just the numbers are much higher because the state's so much larger. In addition, you think that there's about 10 hydropic pregnancies that are dangerous. I mean it's fetal demise plus dangerous for the mom each year. So in theory what states are most highly affected? Well it's currently most related to the percentage of Asians in the state, which is wide ranging. So this is a map of the percent of Asian-Americans in each state, which ranges from about 1% to 15 in California 57% in Hawaii.

So now coming back to the key questions. What's the natural history including the spectrum and severity of deletional, non-deletional HBFH during the first five years. Well much of this I discussed above and there is data lacking to know exactly what's going on how many are actually being diagnosed. There's a wide range of severity of disease but if diagnosed at birth and hemoglobin H Constant Spring about 40% require transfusion before five years of age. If not diagnosed at birth, again we don't have good data. It's this odyssey of you know being diagnosed with anemia and not sure what happens after that potential iron therapy transfusions as I mentioned and the tension with the health care system. In terms of treatment does early identification improve the health of identified children and what treatment methods used? Well how does one quantify the benefit here? Early diagnosis allows the education starting folic acid, screening to transfuse when needed and avoid unnecessary transfusions it leads to avoiding the inappropriate or repetitive diagnostics and treatment with
iron transfusions and it allows that opportunity for genetic counseling and avoiding hydropic pregnancies.

What is the relationship one of that sort of moving down the key questions what's the relationship between treatment outcomes and the timing of treatment? It's not clear. And a lot of this is you must account for the quality of life and the impact of the family, which isn't documented and it's hard to quantify. Why homes are associated with the delay? How does inappropriate iron supplementation affect health outcomes? Well delays lead to the living with anemia the potential need for receiving transfusions that could be avoided. This odyssey of misdiagnosis and inappropriate transfusion and having a more rapid iron accumulation but there's no data in terms of how rapid and what that does down the road. What are the potential harms and risks associated with treatment? Well none- There are no real risks above that other standard risks associated with transfusion.

But here at least you know that the transfusions are being done therapeutically with a high benefit to risk ratio. What are the economics? What are the costs associated with it? Well it's relatively cheap in terms of the testing because it's tapping into the current mandated pipeline. What's the cost effectiveness? It's not known but you know the cost of receiving appropriate care is quite low and you're saving money from avoiding the direct costs of the inappropriate diagnostics and treatments. One of the costs associated with the diagnosis in the failure to diagnose in a pre-symptomatic period well it's much more expensive to diagnose hemoglobin H disease at an older age, because you don't have that advantage of having hemoglobin Barts. And again there's those inappropriate tests that happen on the way to no definitive diagnosis. What's the availability of treatment to lower the costs? Well education cheap. Folate transfusions are all readily available. The key is that newborn screening leads to very efficient access to optimal care as opposed to families stumbling through the medical system before a definitive diagnosis and appropriate treatment. Education, folate and folate are cheap.

Transfusion costs are highly variable but if you're not diagnosed and then transfused [emergently 00:46:14] acute therapy and care is much more expensive. So finally I want to come at this from a different approach. There's been a lot of talk about screening in terms screening for hemoglobin H disease. So what about alpha-thal trait? So as brought up in the first talk, often there's a low normal or widely abnormal hemoglobin and low normal or mildly abnormal MCV. Children with alpha-thal trait are often confused with having iron deficiency anemia. They're often not diagnosed. They're often given iron inappropriately. Often there's the same issue of these diagnostic delays not getting the appropriate testing and repeated courses of iron, allegations of noncompliance and tension with medical providers. And while not nearly as severe as hemoglobin H, it's about 30 to 70 times more common than hemoglobin H.
And it's diagnose is the same newborn screening testing hemoglobin H disease. So you know a side benefit of reporting out hemoglobin H is you could also report out alpha-thal trait, which is what we do in this state allowing for genetic counseling for hemoglobin H disease and Hydrops fetalis. So in Washington state this is a summary of diagnoses in the red box there are hemoglobin H disease and we see in this years only seven.

But then if you look at those with various versions of alpha-thal trait it's far more. It covers a wide range of ethnic background. So I propose reading out on alpha-thal trait in addition hemoglobin H would not be any additional testing costs or at least for this initial screen. In terms of report out in Washington state, it's sending out a letter only. It could include educational information on hemoglobin and iron screening both for the families and the providers along with guidelines for when to refer for genetic counseling. I think it would have a widespread albeit mild impact it would ... there would be more cost of iron screening but this screening cost would be indicated anyway and it would lead to I feel less unnecessary treatment with iron associated followup. And finally of note this is only going to pick up alpha-thal trait not beta-thal trait. And with that I want to thank you for your time and open to questions.

Tim Davis: Thank you Dr Bender.

So the question answer session we can press star 7 to unmute and ask questions over the phone or you can type your questions in the chat box.

Careema Yusuf: Hi Tim. This is [Careema Yusuf 00:49:55], I do have a question in the chat box and it's for Dr. Bender. It says, "You spoke of a hemoglobin Constant Spring and the ability to be observed on gel. Can a pattern for Constant Spring be seen on HPLC. And if so does it have the same retention time as hemoglobin Bart?"

Dr. M Bender: Why don't I defer to Tim because he's far more of an expert on this than myself.

Tim Davis: Yeah we've never seen it on HPLC so I heard that when they're older you can see it but in newborns we don't see it on HPLC.

Careema Yusuf: Thank you. Anybody have a question from the phone you can press star 7 to unmute yourself. We do have another question in the chat box and it says alpha-thalassemia trait reduce the severity of sickle cell disease? Do these patients have a spleen.

Dr. M Bender: So alpha-thal trait is fascinating and complex but alpha-thal trait in the presence of sickle cell disease decreases the severity of some complications and increases others.

Careema Yusuf: Thank you. Any questions on the phone particularly like to ask if so press-?

Dr. Aguinaga: This is Dr. Aguinaga, I have some comments regarding the first question.
They have same rotation time as hemoglobin Bart for hemoglobin Constant Spring and no it is not hemoglobin Bart it's fast [inaudible 00:51:43] hemoglobin so it's going use very [inaudible 00:51:46] and hemoglobin Constant Spring use very far by hemoglobin C. You also tell by the percentage from Constant Spring usually you can see 2%, 1% on the HPLC. On an isolated focus [inaudible 00:52:03] not in an [inaudible 00:52:08] in another patient you can see it have retarded band, very, very top of the gel and it's a very heavy hemoglobin. So it's at the very top of the gel. Whereas Barts is going to be on the other end of the gel as fast running hemoglobin. Thank you.

Careema Yusuf: Thank you Dr. Aguinaga. So just a follow up to that question, what would be the recommended method for Constant Spring detection.

Tim Davis: Can I speak with?

Careema Yusuf: Sure.

Tim Davis: So in Washington what we do is when we get a hemoglobin H disease we use genetic testing so we do an actual mutation test. Sometimes it's seen on the gels and sometimes it's not. So it's not it's not a highly reliable test. The best thing to do is do real time PCR and look for the mutation.

Careema Yusuf: Great thank you. I do have another question in the chat box. It asks what is the difference between hemoglobin Barts and hemoglobin H. The person was under the impression that hemoglobin Barts is seen in newborn screening whereas hemoglobin H is seen in older children.

Dr. Aguinaga: The difference is hemoglobin Barts, it's a tetramer of gamma so it's a gamma four. Hemoglobin H is a tetramer beta it's a beta four. Hemoglobin Barts you can see it at birth and if you see the case that presented of the adult that have hemoglobin H Constant Spring you will soon see some Barts about 7% in that adult. But hemoglobin Barts levels are pretty much use to define different types of alpha-globin gene deletions. When you have hemoglobin H you know you have hemoglobin HPC. Thank you.

Careema Yusuf: Does anybody have a question on the phone you press star 7 to unmute.

Tracy Bishop: Hi this is Tracy Bishop with the California program can you hear me?

Careema Yusuf: Yes we can.

Tracy Bishop: Yeah. We have a question here for Washington. So they're calling out the alpha-thal trait.

Tim Davis: Yes.

Tracy Bishop: And are you providing any follow up testing? We're just suggesting it.
Dr. M Bender: The recommendation in Washington is a recommended CBC and retake later and there's guidelines for monitoring for growth and contact information for genetic counseling or questions.

Tracy Bishop: OK. Just curious because here in California we provide molecular testing for the possible hemoglobin H disease that we call out. But I don't think if we thought about doing trait would be able to manage that for all those so ... interesting idea. Thank you.

Careema Yusuf: Thank you. I do have a question in the chat box that says what other molecular tools used in Washington for second tier screening for hemoglobin H Constant Spring.

Tim Davis: So what we use is this real time PCR to little discrimination, it basically a [inaudible 00:55:43] and if you'd like to contact me I'd be happy to give you the sequence. And in the next presentation I'm also going to talk about it as well.

Careema Yusuf: Thank you. Tim would you like to say a little bit about what's in the next presentation somebody asked in the chat box about what part two of the series will be part.

Tim Davis: In part two it's going to be more of newborn screenings so the first part was the clinical presentation and now it's the newborn screening end, so we're going to talk about ... there's a survey that we did will talk about that. So we need to know what the current status of screening is in the country. We'll also talk about algorithms, we'll look at chain expression again. We'll also look at examples of percentages of Barts. We'll look at isoelectric focusing, we'll look at HPLC as well. So there'll be a lot more coming.

Careema Yusuf: Great. Thank you Tim. Does anybody have a question on the phone if you do please press star 7 to unmute your line. I do have a question in the chat box it asks is stem cell transplantation an option for hemoglobin H and hemoglobin H Constant Spring disease.

Dr. M Bender: Absolutely it's an option not deletion hemoglobin H in general is not severe enough that I think most people will not think about transplanting it hemoglobin H with Constant Spring, it will really depend on the person's course. There're some patients that do great never require a transfusion, those that might require chronic transfusions definitely will be discussed.

Careema Yusuf: Great. Thank you. Anybody else have any questions? Can press star 7 to unmute or you can type your question in the chat box. Okay I'm hearing none. Tim would you like to close out the webinar please.

Tim Davis: Yup. A recording of this webinar will be archived on the APHL newborn screening training web page. You will receive a link to this page in your post webinar follow up email-mail. PACE credits will also be awarded for attending.
this webinar. To receive PACE credits you must complete the post evaluation survey, which will appear at the post webinar pop up window and the follow up email-mail. If anyone has questions you can contact [Careema Yusuf Yousef 00:58:25]. And thank you for joining us. Part 2 will be on June 29 at the same time. Take care.

Careema Yusuf: Thank you.