7UPPRESS
Larry Thompson
National Human Genome Research Institute
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Operator: Good morning and welcome to the Undiagnosed Disease Program press conference hosted by the National Institute of Health. This press conference will last for 60 minutes. There will be four primary speakers who will provide brief remarks and then members of the media will be invited to ask questions. To ask questions, you can press 'star' and '1' on your touch tone phone to enter the queue. You leave the queue by pressing the pound key. This call will be recorded, transcribed and available tomorrow after 5:00 PM Eastern on the websites of the National Human Genome Research Institute at www.genome.gov and the NIH Office of Rare Disease Research. Now I will turn the program over to moderator Larry Thompson, Chief of Communications at the National Human Genome Research Institute. Please go ahead, sir.

Larry Thompson: Thank you very much, John. Good morning, everybody. I am Larry Thompson, Chief of Communications and the Public Liaison Branch at the National Human Genome Research Institute at the National Institutes of Health. On behalf of the National Human Genome Research Institute and the National Heart, Lung and Blood Institute I am pleased to welcome everyone who has joined us for this tele-briefing. Additional materials including videos, x-rays, photos related to this announcement will be available at genome.gov in the newsroom section of the website. Spokespersons for this tele-briefing will include Dr. William Gahl, the Clinical Director of the National Human Genome Research Institute, and Dr. Manfred Boehm, Investigator at the National Heart, Lung and Blood

Institute also at NIH. We are also really happy to have with us Louise Benge and Paula Allen, two patients whose participation in the NIH Undiagnosed Disease Program led to the discovery that we are announcing today. During this morning's tele-briefing we will be referencing the paper that will be published in the February 3rd issue of the New England Journal of Medicine. Please note that the contents of this press briefing and the content of the paper are embargoed until 5:00 PM Eastern, Wednesday, February 2nd. So now let me introduce Dr. Bill Gahl who will put the story of the disease discovery into some context for you. Dr. Gahl?

Dr. William Gahl: Thank you, Larry. I'm Dr. William Gahl and originally from Milwaukee, Wisconsin but now I'm clinical director of the National Human Genome Research Institute at the National Institutes of Health and director of the NIH Undiagnosed Diseases Program. This program is a trans-NIH initiative of the National Human Genome Research Institute, the NIH Office of Rare Diseases Research and the NIH Clinical Center. When we first announced the Undiagnosed Diseases Program in May of 2008 we had two goals: first, to provide hope for patients whose diseases had eluded diagnosis by offering them a chance to have their condition to be evaluated here at the NIH; and second, to conduct fundamental medical research on the causes of diseases. From the start, we expected that this pursuit might lead to the discovery of new diseases and of variations of known diseases. The NIH Undiagnosed Diseases Program provides a multidisciplinary approach that represents the best chance for a diagnosis. The program provides a vehicle by which doctors with different areas of expertise can collaborate.

In just two and a half years, physicians from around the country have sent us 1,700 referrals, often accompanied by thick medical charts. We accepted 330 of those patients into the program. Each person typically comes to the NIH Clinical Center for a week of specialized consultations and testing. For the 15 to 20% of cases that we have successfully diagnosed, whether on clinical, molecular or biochemical grounds, the results have taken from a week to as long as two years. We use the same tools available at other hospitals – x-rays, bone density scans, all the kinds of tests that may have been done before. But in addition we apply advances in genomic analysis that are not yet available at hospitals around the country. We conduct our own genetic screening and analysis within the National Human Genome Research Institute's genomic score. When we have DNA from affected and unaffected patients, we also perform single nucleotide polymorphism or SNP analysis, as we did in this case. For other cases, we've started to perform whole exome sequencing or next generation sequencing to analyze the 1.5% of the human genome that contains functional code including genes.

In the spring of 2009 an application arrived from a physician named Dr. Karen Saylor in Mount Vernon, Kentucky, whose patients, Louise Benge and Paula Allen, were middle aged siblings. Their radiological images astounded us in the program. Their x-rays showed a clear buildup of calcium in the large vessels of their hips, legs and hands. The two sisters and their siblings had endured this painful condition without a diagnosis for 20 to 30 years. Finding the cause of this disorder was a major advance in biology and provided hope for a future treatment. We have discovered the genetic cause of a disease that we call Arterial Calcification due to deficiency of CD73, or ACDC. With the help of collaborators at the

University of California, San Francisco and the John the Baptist Hospital in Turin, Italy and at the Great Ormond Street Hospital in London who share authorship with the New England Journal paper, we located and studied a total of nine patients with ACDC. Fortunately, the breadth of basic and clinical research at the NIH is comprehensive. When we received the records from Kentucky describing the case of an undiagnosed vascular disorder, I conferred with Dr. Manfred Boehm, an NIH physician who studies vascular biology in the National Heart, Lung and Blood Institute. I'd like Manfred to add to my brief reference to ACDC with some particulars from our paper.

Dr. Manfred Boehm: Thank you, Bill, and good morning everybody. As you mentioned, I'm an investigator in the Center for Molecular Medicine at the Heart, Lung and Blood Institute here at NIH. My research focuses on understanding cardiovascular repair and the genetic that underlies this process. I would like to explain briefly the finding of the paper that will be published this week, particularly the molecular and genetic findings. Certainly, the physical manifestation of this disorder presents significant problems for patients with ACDC including pain and limited range when walking, reduced circulation and blood pressure in lower leg. The x-ray identified calcification occurring where it should not. However, this ACDC disorder spares the coronary arteries. We had a key advantage in reaching diagnoses in this case, involvement of many family members who could contribute samples for our testing. We were not only able to look for markers in the DNA of siblings but also from their parents. We analyzed the DNA of Louise and Paula's parents who did not have the disorder, and their siblings who had the disorder. We used nucleotide polymorphism to determine the region in the genome of the siblings that contain the disease-

causing gene. Dr. Cynthia St. Hilaire, a post-doc in my lab who is here with us, can answer specific questions you may have regarding the technique we used in the lab.

As a first step in our approach to study this disorder, Dr. St. Hilaire drew fibroblast cells obtained from tissue samples of the patients. She found significant calcium precipitation in the cells after three weeks in culture. This was important to show that we were not dealing with a problem of circulating calcium but something intrinsic to the cell. At the same time, Dr. Thomas Markello in Dr. Gahl's lab was conducting a genetic analysis of DNA, studying the single nucleotide polymorphism or SNP in the genome of the patients. This study detected a single region of the genome in the sibling in which both strands of DNA in the chromosomes are alike. The region spanned 92 genes. We zeroed in on one gene called NT5E. We found a mutation in each copy of the sibling's genes and only in one strand of DNA of their parents, implying that each parent passed along the mutated copy of the gene variant to the sibling.

The NT5E gene product produces a protein called CD73 which was almost absent in the affected patients in this study. CD73 is important for the production of adenosine, a small molecule produced on the surface of the cell that prevents calcification of the wall of arteries. The reduction of adenosine in turn contributes to an elevated activity of tissue non-specific alkaline phosphatase. This enzyme then creates an inhibitor of calcification called pyrophosphate. In short, deficiency of CD73 leads to this arterial calcification. For those who are interested in the biochemistry, we provide a figure showing the process of increased tissue calcification in figure four of our paper. Through our contacts in the

medical community, we learned about two other families with the same condition. We saw one of the patients at NIH, [unintelligible] participating from Italy where a collaborator conducted a similar test to confirm the condition. Our collaborator found an NT5E mutation that produces nonfunctional CD73 in each of the three individuals in this study. In the coming months we are anticipating offering these patients the option of participating in a clinical treatment study at NIH that is currently being reviewed for approval. This will involve treatment and continuous study. To summarize, the discovery of ACDC has been an exciting step in providing new insights for this patient group as well as a new understanding about vascular biology.

Larry Thompson: So thank you very much, Doctors Gahl and Boehm. Fortunately, we have the sisters here to tell us their stories, both Louise Benge and Paula Allen. I'd like to ask Louise to go first and tell us a little bit about herself and her condition.

Louise Benge: Hello, I'm Louise Benge. My sister, Paula, and I were flown here from Brodhead, Kentucky for appointments here in the NIH Clinical Center and to be a part of this announcement. I began to experience the symptoms of this condition when I was in my 20s. Now I'm in my 50s. So it had slowed me down quite a bit and impacted my life for a long time. At work, my job requires a lot of data entry even with the pain that I have in my hands. It hurts to stand and it hurts to walk, but I keep up with the activities of my family. I am married to Johnny, have a 32-year-old son, Billy. My daughter Suzanne passed away from cystic fibrosis in 1999 when she was just fifteen and a half. I also have two stepchildren, Denise and Josie, and a granddaughter, Miley. None of my doctors until now have

known exactly what cause the calcium buildup in my arteries. A lot of them tell us just to go about our business as usual. They have tried very hard but they haven't had a lot to offer us. That's why we came to NIH. I'm really impressed with everyone connected with the National Institute of Health. It has been a very impressive experience and I'm excited that they found out what was causing our problems and pray that someday soon they will be able to do something about me, Paula, Elizabeth, Daryl and Dad. And even if they can't help us, maybe someday they will be able to help someone who also has this problem.

Larry Thompson: Now I'd like to ask Paula if she could also say a few words.

Paula Allen: My name is Paula Allen. I'm Louise's younger sister. My brothers and sisters and I have some symptoms of the condition also. I'm a senior customer service representative for Kentucky Utilities Company in Richmond, Kentucky. I live in Brodhead with my husband Johnny and I have a son, Tanner, a daughter, Heather, and three grandchildren, Brent, Kaitlin and Trenton. My symptoms started in the calves of my legs. Over a time period the pain got so bad that my sleep was interrupted. At that point I was offered surgery to reroute the artery. I was also told that they would have to redo the surgery in about five years, so I declined on the surgery. Both Louise and I have tried weekly infusions of sodium bisulfate which was to help our kidneys filter out calcium from our blood. It wasn't doing what they had hoped for us, so we're not doing that at this time. My brothers and sisters all came to NIH for tests, so did Mom and Dad. We're all impressed by NIH and I'm excited by the fact that they might find something to help us or to help someone else.

Larry Thompson: So thank you, ladies. Thank you both for being here and for dealing with this condition all these years. So what we'd like to do now is open up the phones for questions. So I'd ask everybody who's going to ask a question to identify themselves and your organization. If you have somebody in particular that you'd like to answer the question, state it. If not, we'll just sort of figure it out as we go along. So, John, would you please bring the first question.

Operator: Absolutely. At this time if you would like to ask a question, please press 'star' and '1' on your touch tone telephones. You may withdraw yourself at any time by pressing the pound key. Once again, to ask a question, please press 'star' and '1'. We will take our first question from Martin Johnson with the Milwaukee Journal Sentinel. Please go ahead, sir.

Martin Johnson: Hi. Thanks very much for making the announcement and taking our questions. I wondered whether you sequenced all of the genes of the patients you looked at or like whether this was either whole genome or whole exome sequencing or another technique?

Larry Thompson: So, Dr Gahl?

Dr. William Gahl: Well, it turns out that the SNP array analysis pointed to a region that had 92 genes in it, and we knew that this was going to be a recessive disorder essentially because the parents were third cousins and so it made sense. So we used the model in which we expected that all five of the affected individuals, the siblings, would be homozygous and the parents would be heterozygous. So we used the million SNP array analysis to find that region, and there was only one region, and that encompasses 92 genes and

it turns out that three of the genes were pretty good candidates. So we looked at those for expression and Dr. St. Hilaire found that one of those three genes had very poor expression, so we went after that. This all obviated the need to do whole exome sequencing or massive sequencing. So in this case we didn't have to do that.

Martin Johnson: Thank you.

Operator: We would take our next question from Ferris [unintelligible] with New Scientist Magazine. Please go ahead.

Ferris: Hi, I'm Ferris from New Scientist. I was wondering if you could tell us a little bit about your ideas for potential therapies at this point, now that you have a specific molecular target and which one of those molecular steps would be most effective to intervene?

Dr. Manfred Boehm: So, by using cell system models we identify two main therapeutic targets. One was adenosine and the other one is alkaline phosphatase activity. So targeting adenosine is quite complex and there are currently no clinical therapies out to target adenosine signaling phosphate directly at the receptor level. Alkaline phosphatase, there is not a direct inhibitor on the market that inhibits alkaline phosphatase. However, there are certain compounds that have been shown to be effective in other diseases that are related to the disease that we discovered that causes this painful condition in patients. One of these diseases is GACI. GACI stands for Generalized Arterial Calcification in Infants. This is a horrible disease that leads to calcification early on in life, shortly after birth, and these kids they die of myocardial infarction. There has been some improvement in the

prognosis of these kids by using a certain drug called bisphosphonates that has a lot of different effects but can inhibit alkaline phosphatase activity. So it also turns out that this bisphosphonates which is used in osteoporosis quite frequently, and osteoporosis is associated with atherosclerosis. So people were looking, is the effect of bisphosphonates on calcification in atherosclerosis and although our disease is clearly distinct from atherosclerosis, there are some indications that bisphosphonates positively regulate calcium accumulation in arteries. So you can think about that if you have osteoporosis your calcium moves out of the bone, goes to the artery and causes calcification. Bisphosphonates basically reverse this process, although this is a very simple and probably not really scientific accurate description of this process, but it gives you a picture of how this thing, this could work.

Ferris: Okay, thank you.

Operator: Just a reminder, to ask a question it is 'star' and '1' on your touch tone telephone. We will go next to Donna Young with Scrip News. Please go ahead.

Donna Young: Thank you for taking my call. I also wanted to ask a little bit more about the potential therapies. You did explain the bisphosphonates, but does that mean at this point you will not pursue anything on the adenosine side for your trial? Then also, how many patients do you expect to enroll and how soon do you think you can potentially get underway with the first trial in this area? Thank you.

Dr. Manfred Boehm: So, in regard to treatment of patients, we are focusing, we are zooming in on bisphosphonates at the current time. However, there are experimental drugs out on modulating adenosine as well as also new inhibitors of alkaline phosphatase. So we will continue in testing these drugs in in vitro models to find out how they directly affect the calcification. So we are continuing on other leads to find the best drug for the patients. So at the moment we have nine patients identified with these conditions. However, we hope that the release of the publication as well as your report will lead to more people coming in and come to the NIH so we can include them in addition to our study.

In regard to when do we want to start it, so at the moment we are in the process of putting a protocol altogether and it has to go through certain regulatory hurdles, IRB, FDA and probably other institutional hurdles to make sure that this proposed treatment strategy is safe for the patients. So it's a little bit hard to predict but we think hopefully in the next three to four months that we have some idea in regards of the IRB where we stand if we can go forward.

Dr. William Gahl: I would add that it's certainly a lot easier for us to treat with an FDA-approved drug like bisphosphonates than it is for us to treat with adenosine receptor agonists which are not yet approved by the FDA.

Donna Young: Do you have any pharmaceutical partners at this point that you will be using in the trial?

Dr. William Gahl: No, we don't, but again if this is an FDA-approved drug, we may not at least initially need such a partner. This in part depends upon whether

we're required to get investigation of new drug approval by the Food and Drug Administration. Sometimes when you apply for those you don't actually – the FDA will say, "No, you don't need those for an approved drug used off-label."

Donna Young: Thanks very much.

Operator: Next we will go to the site of Tracy Vence with Genome Technology Magazine. Please go ahead.

Tracy Vence: Thank you for taking my question. Dr. Gahl, at the NIH Research Festival on October you spoke a bit about the study which I believe was ongoing at the time. I'm wondering now, what are the implications of your results in terms of learning more about sort of the underlying biology of bone formation and calcification elsewhere in the body?

Dr. William Gahl: Well, this really opens up new avenues for that study, that is to say results have not been obtained yet because we're concentrating on this particular disorder. I think that Manfred may want to speak to this a little bit, too, but there could be applications for calcification elsewhere in the vascular system. I think the fact that adenosine is involved in essentially suppressing calcification in certain cells is a new finding for the vascular biology community, and Dr. St. Hilaire may want to add something to that, too, because she presented these in a vascular biology meeting to a lot of excitement. So I would say that this opens up avenues that really the vascular biologists are going to have to carry. Would you like to say anything?

Dr. Manfred Boehm: Yes. So, I've already gone to how this may impact other bone diseases. In fact, we are already discussing this with folks in the bone research community like Michael Whyte from the Shriner Institute in St. Louis or other endocrinologists who work on bone metabolism. So there are actually two other diseases, one is called Albright hereditary osteodystrophy or AHO and Progressive osseous heteroplasia called POH. These are bone diseases that have in common that they also develop heterotopic calcification, however not dominantly in [unintelligible] in other tissues. So it turns out that the cause for these two diseases is [unintelligible] on something that regulates cyclic AMP. So this is a central molecule in the cell that has a lot of functions. So adenosine also regulates cyclic AMP or actual signals through cyclic AMP. So we try to find common mechanisms by comparing these three, actually four different diseases to find out commonalities between them to identify what leads to the calcification. Maybe Dr. St. Hilaire can talk more about how adenosine regulates cyclic AMP in other pathways.

Dr. St. Hilaire: Yes. So adenosine signaling is well-known in the vasculature and there are many labs that are working it out. Adenosine signals through binding to one of four adenosine receptors and two of those receptors produce cyclic AMP and two of those receptors inhibit cyclic AMP. So we're currently working on figuring out what receptor we think is involved in ACDC. But in terms of the vascular system in general, adenosine signaling has known to be protective. It's also often called the retaliatory metabolite that reacts to protect with muscle cells under stressful conditions. So this finding in protecting the vasculature from calcification is really very new to the whole vascular biology field.

Larry Thompson: Okay, any follow up or the next question?

Operator: We go to the site of Martin Johnson with the Milwaukee Journal Sentinel. Please go ahead, sir.

Martin Johnson: Hi, thanks for taking another question from me. First, I wondered if we could get the spellings and the ages for the two sisters, the patients who've been speaking to us.

Larry Thompson: Ladies, you can tell them your age if you want to but you don't have to. Louise, could you just give them the spelling of your name please?

Louise Benge: Louise is spelled L-O-U-I-S-E, Benge is B-E-N-G-E and I'm 56 years old.

Martin Johnson: Thank you very much.

Paula Allen: Paula Allen, P-A-U-L-A A-L-L-E-N, I'm 51.

Martin Johnson: Okay, thank you very much. I wondered if someone could also speak to what the implications from the study are for the numerous other cases, I guess 200 or other cases currently in the Undiagnosed Diseases Program. Does this open up new avenues for results there?

Dr. William Gahl: Well, a success like this always buoys us in terms of sort of the emotions and it makes people understand that we can actually achieve successes in finding new diseases. That is one of the goals, so that's sort of generic. In addition, there are a couple of other individuals who have calcification in vessels elsewhere, and we're going to apply this new knowledge to them

as well. I think more broadly it indicates that million SNP array analysis can be beneficial but not for every patient. In other words, there are families for whom this is applicable, and every time we see a family like this, and every time we see a new disorder, we learn more about what techniques we should apply to that particular family. So I think we've gotten a lot of general knowledge and as you mentioned about adenosine, some specific knowledge that can actually be applied to a couple of other patients.

Larry Thompson: That was Dr. Gahl speaking. I have to probably remind you guys to identify yourselves because they can't see you.

Martin Johnson: Thanks so much.

Operator: It appears that we have no further questions at this time.

Larry Thompson: Okay, Dr. Gahl, do you have closing remarks?

Dr. William Gahl: No, I was just going to ask.

Larry Thompson: You were going to ask a question? Okay.

Dr. William Gahl: Well, okay, let me ask Dr. Boehm how this applies to atherosclerosis. Is there a possibility that some of the therapies that we're developing here could be applicable to a more common disease? Before I do that, let me answer a question of my own. Why are we studying these rare diseases in this program? Well, it's really because a lot of what we learn about a rare disease is applicable to a common disorder, and that's really why I'm

introducing Dr. Boehm to answer that question. In addition, probably everyone in the audience recognizes that there's someone for whom a rare disease touches that person or a family member or some acquaintance. I think that's an important issue to remember for people who are really supporting the Office of Rare Diseases Research. In addition, patients who have rare diseases are often abandoned by the medical community because we still don't know how to treat if we don't know a diagnosis. The way that our society treats individuals who are abandoned or not receiving the best of medical care is really a measure of our society. So I think that this is a program that really speaks to our society, treats the poorest among us, and that's really another goal of the NIH Undiagnosed Diseases Program. Manfred?

Dr. Manfred Boehm: So, the disease ACDC that we identified is clearly different as I already mentioned from atherosclerosis. So atherosclerosis is a process that is probably initiated by damage of endothelial cells that then leads to her process that involves inflammation, but one of these processes leads to calcification as some kind of dystrophic reaction to the overall [unintelligible] process. The ACDC is different. The calcification does not occur on the same location as atherosclerosis. It occurs in the wall of the blood vessel and it's a kind of regulated process where the calcium is built up in the vessel wall.

So what we learned from this disease that adenosine is linked to this process. Adenosine is widely known, as Dr. St. Hilaire already indicated, in vascular homeostasis. Now what we found is that adenosine through alkaline phosphatase modulates calcifications. So this might have not direct mechanistic implication of atherosclerosis but the treatment strategy

that we test in this disease could potentially be translated also to patients with a much more common disease like atherosclerosis. It may also give us some hints how this connection between osteoporosis in women and their increased risk of developing cardiovascular diseases are linked. Maybe levels of adenosine may slightly modulate this disease or this process.

Larry Thompson: Alright. Well, thank you very much, ladies and gentlemen. Let me give the reporters one last chance to ask a question if they want. Is there anything in the queue, John?

Operator: Actually, we do have a question from Donna Young again with Scrip News. Please go ahead, ma'am.

Donna Young: Alright, thank you. With the bisphosphonates will you be testing oral bisphosphonates or the infusion or both or do you know yet kind of what path you're going to take with those?

Dr. Manfred Boehm: So, we have developed this coming protocol as a team effort within the NIH that develops people like bone basic scientists with some clinical knowledge but also clinicians at the Clinical Center and geneticists like Bill Gahl. So we are debating at the moment what is the best way to do it, but one what we always keep in mind is what is the easiest way for the patients. So of course we know what is – is it easier to take a pill or is it easier to take an infusion? So at the moment if we don't hear from our clinical colleagues who prefer one or the other, we would go for the easiest way, probably a pill a month or something or a pill a day.

Donna Young: Thank you.

Larry Thompson: Alright. Now are there any other questions in the queue?

Operator: Actually, we do have another question from Martin Johnson with the Milwaukee Journal. Please go ahead, sir.

Martin Johnson: Hi. I just wondered if you could clarify a little bit, I'm sorry, the application, how this might help with atherosclerosis, how this might help in terms of either learning more, developing treatments.

Dr. Manfred Boehm: So, as you know, there is a link between atherosclerosis and osteoporosis. So women – I'm just repeating what I've just said.

Larry Thompson: Well, just repeat it but shorter.

Dr. Manfred Boehm: Okay.

Martin Johnson: I'm sorry. I just had difficulty understanding.

Dr. Manfred Boehm: To understand me, yes, actually I have an accent, yes. Okay, so women with osteoporosis, specifically if they develop osteoporosis at a younger age have a higher risk of developing cardiovascular disorders, atherosclerosis. So there's a link between bone remodeling and vascular calcification of atherosclerosis type. So we now identified that adenosine regulates a key player in bone remodeling but also in heterotopic tissue calcification which is alkaline phosphatase. This probably plays a role in atherosclerosis and osteoporosis. So by identifying the exact pathway we

may obtain more knowledge about the connection between osteoporosis and atherosclerosis, which will also lead us hopefully to a more targeted treatment for the one component versus the other.

Martin Johnson: Thank you.

Larry Thompson: Alright, any other questions in the queue, John?

Operator: It appears we have no further questions.

Larry Thompson: Alright. Well then, I'm going to bring this to a close. This is Larry Thompson. I want to thank everybody for participating in today's tele-briefing especially Louise and Paula, thank you for being here and for sharing your stories. We very much appreciate that. Reporters who have any other questions can certainly call the Communications Shop at the Genome Institute where Ray McDougall whose contact information is on the advisories for today's briefing would be happy to assist. We all have been placing up on genome.com's newsroom sound bites from interviews with several of the participants here including Louise and Paula. Hopefully we'll have those up later today. We'll get the audio file of this also up as quickly as we can, and there's other background information in the newsroom. So there should be lots of assets to help you tell your stories, but if you have any other questions you can certainly go ahead and give us a call. With that, I'd like to bring this tele-briefing to a close and thank everybody for your participation. Thank you very much.

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