The New York Times

Health

Medical Detectives Find Their First New Disease

By GINA KOLATA

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Brendan Smialowski for The New York Times

Paula Allen's legs and feet were checked for blood flow on Tuesday in a clinic in Bethesda, Md. She and her two sisters and two brothers have all suffered from similar pain in their extremities.

Louise Benge's medical problems started when she was 25. Walking became excruciating. Her calves got hard as rocks, and every step was agony. Her hands started hurting too. And the condition, whatever it was, only got worse over the next two decades.

Ms. Benge's family doctor in Mount Vernon, Ky., was at a loss, as were a vascular specialist, a hand specialist and a kidney specialist. Her two sisters and two brothers had the problem too, but no doctor could figure out why.

It was clear from X-rays why Ms. Benge could barely walk: The blood vessels in her legs, feet and hands were accumulating calcium deposits like the scale that sometimes forms inside water pipes. The deposits had grown so thick that blood could hardly squeeze through. But calcium was only in those blood vessels of her legs and hands; her heart's vessels were spared, so she was not in immediate danger of dying.

A doctor prescribed weekly infusions of a drug, sodium thiosulfate, Ms. Benge said, thinking it might bind to the calcium so her body could flush it out. But the drug did not work — it only made her vomit.

Finally, Ms. Benge's family doctor sent her medical history to a detective agency of sorts, the Undiagnosed Diseases Program at the National Institutes of Health. Set up in the spring of 2008, the program relies on teams of specialists who use the most advanced tools of medicine and genomics to try to figure out the causes of diseases that have baffled doctors.

The idea was that understanding rare diseases can give insights into more common ones, said Dr. William A. Gahl, director of the program.

And, he said, there was another reason.

"Patients who have rare diseases are often abandoned by the medical community," Dr. Gahl said. "We don't know how to treat if we don't have a diagnosis. The way our society treats abandoned individuals is a measure of our society. It speaks to how our society treats the poorest among us."

With Ms. Benge and her siblings, the researchers have their first newly discovered disease. It is caused, they report on Thursday in The New England Journal of Medicine, by a mutation in a gene that prevents calcium from depositing in blood vessels.

Now that they know the cause of the disease, the researchers have ideas for how to treat it. And the discovery also has implications for more common diseases, like heart disease and osteoporosis, in which calcium is deposited inappropriately.

The unraveling of Ms. Benge's mystery disease began the week of May 11, 2009, when Ms. Benge, who is 56, and her sister Paula Allen, who is 51, arrived at the tall red-brick clinical center on the campus of the National Institutes of Health.

The Office of Undiagnosed Diseases had been hearing from thousands of patients, Dr. Gahl said, 1,700 of whom sent their medical records. "Many had been to Hopkins, the Mayo Clinic and the Cleveland Clinic, and some had been to all three and been there more than once," he said.

Dr. Gahl and his colleagues were looking for people with unusual symptoms or unusual clues to what might be wrong. For example, they are now investigating a mystery disease in a young girl with uncontrollable muscle contractions that make it hard for her to talk, walk and use her hands; one that gave a young boy symptoms that look like Parkinson's disease; and one that gives a middle-aged woman shards of keratin, a hair protein, coming out of her hair follicles.

Ms. Benge and her sister had symptoms like no one had ever seen before. X-rays and M.R.I. images of their legs, hands and feet showed blood vessels so clogged with calcium that blood could get through only by squeezing into tiny vessels that had sprouted to circumvent the blockages. And those tiny vessels just were not able to supply enough blood.

Because there were five affected siblings, the researchers decided to take a genetic approach, using techniques not available at most major medical centers. The parents were fine, and that indicated the disease might be caused by a recessive gene — each parent would have one copy of the mutated gene and one copy of the intact gene, and each child with the disease would have two copies of the mutated gene, one inherited from each parent.

That led the investigators to a stretch of DNA with 92 genes. From there, the researchers zoomed in on the gene that was the culprit. A mutation had stopped it from functioning.

Cells use the gene to make extracellular adenosine, a common compound that, in this case, was needed to suppress calcification. No one had known about this metabolic pathway, said Dr. Manfred Boehm, a vascular biologist at the National Heart, Lung and Blood Institute.

The discovery is very important, said Dr. Dwight Towler, a bone endocrinologist at Washington University in St. Louis who was not part of the study, because it can help researchers understand signals for calcification in different parts of the body.

"You notice they don't have problems everywhere," he said of Ms. Benge and her siblings. That is because bone calcification and blood vessel formation are exquisitely coordinated, and different parts of the body use similar, yet subtly distinct, mechanisms.

The disease also fits in with a growing understanding of the close relationship between blood vessel cells and bone cells. Researchers say it could lead to new insights into heart disease, in which calcium deposits in coronary arteries, and heart valve disease, in which calcium can deposit in heart valves. Sometimes, said Dr. Towler, actual bone, with marrow, forms in valves.

It also could help illuminate the relationship between osteoporosis, in which bone is lost, and heart disease. In osteoporosis, as people lose bone, calcium often accumulates in arteries. It is as if the calcium that is not being deposited in bones is going into blood vessels instead.

"I think it has to do with the fact that the cells that make up our blood vessels are of the same origin as the cells that make up bone," said Dr. Gahl, who is also clinical director of the National Human Genome Research Institute.

The researchers have now identified nine people from three families who have the newly discovered disease: Ms. Benge's family, a patient in San Francisco and a family in Italy. Now they are working on treatments. The simplest might be to give a bisphosphonate, an osteoporosis drug. With the gene mutation and decreased levels of adenosine, patients end up with high levels of an enzyme, alkaline phosphatase, needed to make calcium deposits. Bisphosphonates bring down levels of that enzyme.

The investigators are putting together plans to test bisphosphonates and submitting them to ethics boards for approval.

"We hope to know in three or four months whether we can go forward," Dr. Gahl said.

Los Angeles Times

Researchers find gene at root of rare hardening of arteries

By Thomas H. Maugh II, Los Angeles Times
February 3, 2011 12:16 p.m.

National Institutes of Health researchers have discovered a gene that causes a rare but extremely painful buildup of calcium in the arteries of legs and hands, the first mystery that has been solved in the NIH's Undiagnosed Disease Program. Although researchers have so far identified only nine patients with the ailment, they suspect that identifying the cause of the problem will provide insight into much more common disorders.

The NIH launched the program in 2008 in an effort to help patients for whom all other attempts at diagnosis have failed. The program brings together NIH scientists and outside specialists who use the most advanced tools available to try to explain the previously inexplicable. "People who have rare diseases are often abandoned by the medical community," said Dr. William A. Gahl, director of the program. "We don't know how to treat if we don't have a diagnosis. This is a program that speaks to how society treats the poorest among us." So far, the program has received 1,700 referrals and accepted 330 patients, who come in for a week of specialized testing.

In the spring of 2009, Dr. Karen Saylor of Mt. Vernon, Ky., referred sisters Louise Benge, 56, and Paula Allen, 51, to the program. Benge developed symptoms in her 20s and can now barely walk. Calcium deposits have accumulated in the blood vessels in her legs and hands to the point where almost no blood can flow through. Her calves are rock hard. Saylor tried administering sodium thiosulfate to flush out some of the calcium, but it only made Benge sick. Allen's symptoms were only slightly less severe. Two younger sisters and two younger brothers had earlier stages of the disease. When the NIH researchers took X-rays, "the radiological images astounded us," Gahl said. The arteries had so much calcium, they looked almost like bones.

Surprisingly, however, arteries in the chest and the rest of the women's bodies were normal, so they were in no immediate danger of dying from atherosclerosis.

Because all six children had the disorder, but neither parent -- who are cousins -- the researchers suspected a recessive genetic disease. That is, they suspected that each parent carried one copy of a defective gene and, through extreme bad luck, each child had received the bad gene from both parents. Analyzing DNA from the parents and the children, they quickly focused on a sequence of 92 genes that carried what they suspected to be the defective gene, said Dr. Manfred Boehm of the National Heart, Lung and Blood Institute.

Further study quickly narrowed the search to a single gene, called *NT5E*, the team reported Thursday in the New England Journal of Medicine. This gene normally makes a protein, called CD73, which is

important in the production of adenosine. Adenosine, in turn, protects arteries from calcifying. The team has since identified the same defect in two other families, for a total of nine patients. "We hope the publication of this paper will lead to other patients coming in," Boehm said.

The team is now trying to devise a treatment program for the patients. One possibility is to use bisphosphonates, which are used to prevent osteoporosis. The team is now attempting to get a treatment protocol approved by their ethics board. There are also experimental drugs that might alter adenosine metabolism, Gahl said, but developing a protocol for them will be more difficult than for the bisphosphonates, which are already approved by the Food and Drug Administration.

Louise Benge said she and her sister are "very excited that they found out what is causing this. ... Maybe someday they will be able to help someone with this problem."

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Researchers use genomic study to discover new disease

Finding raises hope for treating illnesses

By Mark Johnson of the Journal Sentinel

Feb. 2, 2011

In her 20s, Louise Benge began experiencing mysterious leg pain. MRIs and X-rays of the Kentucky woman's blood vessels revealed the source of the problem: calcium deposits in the artery walls. But doctors could not explain why the calcium was building up, restricting blood flow until it hurt to walk or even stand.

The mystery deepened as her younger sister, Paula Allen, began suffering leg pains so severe she struggled to sleep at night.

This week researchers working for a special program at the National Institutes of Health announced that they have found an answer encoded in the women's genetic script.

The achievement, reported in Thursday's New England Journal of Medicine, lends support to those pushing to bring genomic medicine into the hospital and boosts hopes for the NIH Undiagnosed Diseases Program, a kind of "cold case" unit formed to investigate illnesses that have stumped doctors. Since its launch in 2008, the program has decided to investigate the cases of 330 patients with mysterious illnesses; so far 35 have been diagnosed.

The NIH study follows a similar report last year describing how researchers at the Medical College of Wisconsin and Children's Hospital of Wisconsin tracked the source of young Nicholas Volker's intestinal disease to a single mutation. The information in the child's DNA helped doctors diagnose and treat him.

In the Kentucky cases, NIH scientists scanned portions of the women's genome and discovered that they have a new disease. Scientists named the disease arterial calcification due to CD73 deficiency, or ACDC. CD73 is a protein that protects arteries against calcification.

The new finding lends support to those pushing to bring genomic medicine into the hospital and boosts hopes for the NIH Undiagnosed Diseases Program, a kind of "cold case" unit formed in 2008 to investigate illnesses that have stumped doctors. So far the program has decided to investigate the cases of 330 patients with mysterious illnesses; 35 have been diagnosed.

NIH officials said seven other patients have the same disease as Benge and Allen, who are now 56 and 51 respectively. All of the patients come from three unrelated families.

William A. Gahl, who directs the Undiagnosed Diseases Program and is one of the authors of the study, said the work shows that scientists can find and explain new diseases. However, he said, the study also has implications beyond the world of rare diseases.

"A lot of what we learn about a rare disease is applicable to a common disorder," Gahl said, explaining that discovery of the new disease could lead to improved understanding of the more common ailment atherosclerosis, or hardening of the arteries.

"I think it's an extremely significant finding," said Nicholas Katsanis, director of the Center for Human Disease Modeling at Duke University. Katsanis did not participate in the NIH study.

"They were a little bit fortunate, but fortune favors the brave."

Katsanis and other scientists stressed that DNA-reading technologies may not always solve the mysteries surrounding diseases. So far about a quarter of the cases investigated by the NIH program have been closed without a diagnosis.

Katsanis said the NIH team was fortunate in finding a pattern of inheritance for the new arterial disease, but added, "to their enormous credit," the researchers then went back and showed that variations in the gene NT5E led to the protein deficiency, which resulted in calcium buildup in the women's arteries.

While the doctors in Wisconsin sequenced parts of all 21,000 of Nicholas Volker's genes, the NIH scientists used a different technique analyzing what are called single nucleotide polymorphisms or SNPs, individual sites in the genome where a person's sequence varies from the norm.

"It's a broad-brush approach, but you basically query landmarks scattered throughout the genome," Katsanis explained.

Researchers learned that the parents of the two Kentucky women were unaffected by the disease. They suspected a recessive inheritance in which children must receive one copy of the gene variation from each parent in order to get the illness.

Using this information, the scientists looked for places in the genome where the sisters received two copies of a DNA segment, but both parents only received a single copy. Their analysis turned up only one region that fit this description, a variation in the gene NT5E. This gene is supposed to make the protein that protects arteries from calcifying.

Scientists then looked at what happens to cells that have this gene variation. They found that these cells, lacking the protective protein, were unable to inhibit the calcifying process.

So far, the Kentucky women have not received a therapy that eliminated their pain, but the NIH researchers said they are now using what they have learned about the disease to search for an effective treatment.

JSOnline.com To read the Journal Sentinel's special report on how physicians and researchers tackled the mystery of Nicholas Volker's illness, go to www.jsonline.com/dna.

nature news

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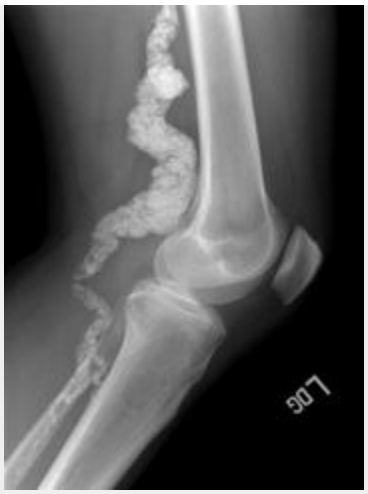
News

Solution to medical mystery offers treatment hope

Mutations underlying a rare disease suggest therapeutic options for common conditions.

Heidi Ledford



A treatment for a rare artery-hardening condition could lead to cures for other more common conditions.*NHGRI*

An effort to discover the causes of mystery illnesses has borne fruit. A team from the Undiagnosed Diseases Program at the US National Institutes of Health (NIH) in Bethesda, Maryland, today reveals the genetic mutation behind a rare artery-hardening condition — a finding that could help to treat more common maladies with similar symptoms.

For Louise Benge the mystery began over twenty years ago, when her legs began to hurt. Now it is part of her daily life. It hurts when she stands. It hurts when she walks. X-rays revealed that arteries in her legs were hardening, but doctors couldn't explain why.

What was clear, however, was that Benge was not alone: her four siblings had the same problem. The pain was so severe that not one of them could walk for more short distances. "It has slowed me down quite a bit," says Benge. Her sister, Paula Allen, says that her own pain sometimes keeps her up at night.

Today, in the *New England Journal of Medicine*[1], the NIH team announces that it has at last found the source of Benge's agony. A mutation in a gene called *NT5E* is plaguing her family tree, causing calcium deposits in her arteries.

NIH researchers are already preparing a clinical trial to treat the condition. They are optimistic that the results could help them to develop treatments for diseases such as atherosclerosis, in which arteries become hardened and clogged, leading to heart attacks and strokes.

"It shows the power of these very rare genetic diseases for helping us understand basic biology," says Michael Bamshad, a geneticist at the University of Washington in Seattle, who was not involved with the study. "There are probably hundreds, if not thousands, of syndromes with the same potential that await discovery."

Concerted effort

The NIH launched the Undiagnosed Diseases Program in 2008 (see Last chance clinic). Since then, the program has accepted 330 patients from among the 1,700 referred to it by physicians around the United States. The patients visit the NIH campus in Bethesda, Maryland, for a week of specialized consultations and testing.

In 2009, a referral arrived from a physician in Mount Vernon, Kentucky. Her patient, Benge, had leg, buttock, and foot pain, but it was her misshapen, hardened arteries that caught the team's attention. "The X-rays astounded us," says William Gahl, director of the Undiagnosed Diseases Program and a co-author of the study.

Gahl and his colleagues collected DNA from Benge, her siblings and four other people with similar symptoms, as well as Benge's parents, who do not have the disease. By testing a million genetic markers, the team pinpointed a region of the genome that was mutated in each patient.

The region contained several genes, but the researchers zeroed in on *NT5E* because they reasoned that the protein it encodes, called CD73, could have a role in calcification. Their gamble paid off: all the people with the disease produced very low levels of CD73, and had mutated *NT5E* genes.

Treatment trial

The results are exciting, says Linda Demer, who studies calcification in atherosclerosis at the University of California, Los Angeles. Demer notes that CD73 helps to break down an important cellular chemical called adenosine monophosphate (AMP). Loss of CD73 could cause a build-up of AMP, high levels of which have been linked to hardened arteries, she says.

Now, less than two years after Benge's case arrived at the NIH, the team is awaiting approval from ethics committees to test a class of drugs called bisphosphonates on her and the other patients. Bisphosphonates have been used to treat babies with a similar condition, generalized arterial calcification of infancy, which is caused by a mutation in the cellular pathway that is affected by CD73.

If successful, the treatment could potentially be extended to patients with similar, but much more common, conditions, says Manfred Boehm, who studies diseases of the veins and arteries at the National Heart, Lung, and Blood Institute in Bethesda, and also worked on the project. Ending the suffering of one family could help millions.

POPULAR SCIENCE THE FUTURE NOW

National Health Detective Squad Uses Genomic Tools to Diagnose its First Mysterious Disease

By Clay Dillow Posted 02.03.2011 at 4:44 pm

Medical detectives National Institutes of Health have just cracked their first case wide open, a result they hope to repeat with a slew of other uncharacterized illnesses and conditions. The Undiagnosed Diseases Program (UDP), a sleuthing agency set up within the NIH in 2008 to connect the dots between cases of undiagnosable illnesses, has traced the source of an extremely rare vascular disorder back to its genetic roots, notching the first closed case for the UDP and another victory for diagnosis genomics.

The case: a rare and debilitating buildup of calcium in the below-the-waist arteries and joints of a small sample of individuals; only nine individuals from three different are known to have the disorder. The likely culprit: a faulty gene, particularly one that may involve recessive inheritance, in which offspring receive copies of a gene variant from each parent that only cause disease when combined (this is why the parents don't suffer from the disease but, in the case of one family, all five offspring do).

So the UDP got down to some genetic gumshoeing, analyzing DNA from the entire family and zeroing in on those places where the sibling had two copies of a particular DNA segment but the parents had only one. They isolated one such region, then cross referenced that against 200 genomes not afflicted with the condition. Doing so allowed them to find the common thread among the siblings: a variant in a gene known as NT5E. Sure enough, the other few reported cases of the condition reported the same variant at NT5E.

The pieces of the puzzle then began falling into place. People with this variant in the NT5E gene have elevated levels of TNAP, an enzyme that is key to tissue calcification. TNAP also degrades an inhibitor of calcification called pyrophosphate that is the body's natural fighter of arterial calcification. Without the pyrophosphate and with TNAP running high, the arteries and joints were experiencing higher levels of calcification, earning the condition the rock-and-roll moniker ACDC, or arterial calcification due to CD73 deficiency.

Case closed (almost). Researchers are still trying to figure out why this only happens in arteries and joints below the waist (it could have something to do with the distribution of certain receptors in the body). But the diagnosis shows the value of a new kind of medicine that aims to ferret out the causes of even the rarest conditions, a process that doesn't just help a select few patients but benefits our body of medical knowledge as a whole.

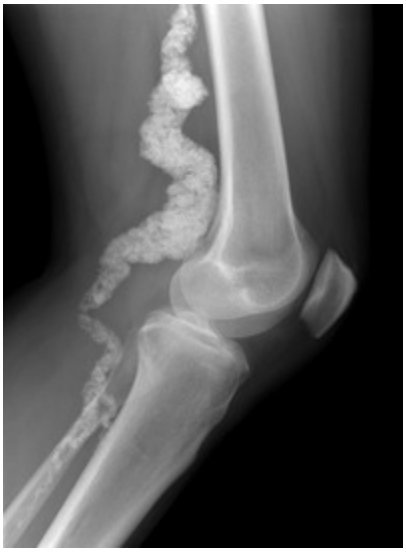
It also further shows the worth of genomic tools as instruments of diagnosis and not just research. There was no Dr. House moment here where it turned out the strange symptoms were caused by the patient's secret methadone habit combined with a misdiagnosed case of syphilis and a peanut allergy. To truly understand the root causes of rare disorders, we have to dig straight into the biological blueprints.

ScienceInsider
Breaking news and analysis from the world of science policy

NIH Undiagnosed Diseases Program Uncovers Its First New Genetic Disorder

by Sara Reardon on 2 February 2011, 5:00 PM

A National Institutes of Health (NIH) program aimed at tracking down the causes of mysterious diseases is bearing fruit. A multidisciplinary team of physicians and genetic researchers have discovered the genetic cause of a bizarre vascular disease afflicting two sisters that leads to a buildup of calcium in the arterial walls, causing joint hardening and pain.



The group's paper, published in tomorrow's issue of *The New England Journal of Medicine*, is the first report of a new genetic disease to come out of NIH's 3-year-old Undiagnosed Diseases Program. It's a consortium of researchers from across NIH and volunteer experts from academic medi cal centers around the country who receive referrals for patients whom physicians have been unable to diagnose. "A success like this buoys us emotionally and encourages us," program director and study co-author William Gahl, who is also clinical director of the National Human Genome Research Institute, said in a press conference Tuesday.

Since its inception in 2008, the Undiagnosed Diseases Program has received 1700 referrals and accepted 330 of them; the researchers report seeing 150 to 200 patients per year. The program's aim, Gahl said, is twofold: to diagnose patients whose diseases have eluded medical knowledge and to conduct research.

In 2009, the program received a referral from a Kentucky doctor for two sisters, Paula Allen and Louise Benge, who suffered from joint pain and showed calcium buildup in their arteries in x-rays (above). The images "astounded us," Gahl said. The team obtained DNA samples from the sisters and other family members (three of Allen and Benge's siblings had the same recessive disease) and scanned the DNA for markers called single nucleotide polymorphisms that the researchers used to narrow the location of the disease gene. By also examining this genetic region in two other families with similar disorders, the researchers were able to pinpoint a mutation in a specific gene, *NT5E*, which is involved in breaking down calcification in the arteries.

The researchers named the disease ACDC (arterial calcification due to CD73 deficiency). The discovery revealed a previously unknown molecular mechanism for preventing calcification and may open treatment avenues for patients suffering from the more common diseases of atherosclerosis and osteoporosis, said study senior author Manfred Boehm of NIH.

Allen and Benge praised the "impressive" work by NIH. "Even if they can't help us, maybe someday they can help someone else with these problems," said Benge. Boehm said that a clinical trial for a drug to treat the sisters and others with ACDC is currently under review by an ethics board.

The undiagnosed diseases program is "wonderful; ... we were thrilled when it started," said Mary Dunkle, spokesperson for the National Organization for Rare Diseases (NORD), a Washington, D.C.-based patient advocacy group. NORD has referred a number of patients to NIH. "In our world, a lot of times knowledge is gained in small increments," Dunkle said. "It's always progress when they're able to identify something that's a complete new entity."

**This item has been corrected 4 February to reflect that study co-author William Gahl is also the clinical director of the National Human Genome Research Institute.*

NewScientist

02 February 2011 Magazine issue 2798

Undiagnosed Diseases Program finds rare new disorder

AN 18-month wait for a diagnosis might seem extreme, but not when the medical disorder in question was formerly unknown.

In 2008, the US National Institutes of Health established the Undiagnosed Diseases Program (UDP) to help people with mysterious conditions. This week it announced its first big discovery: the genetic and molecular basis of a previously unexplained condition that causes painful calcification of the arteries.

Currently, only nine individuals are known to have the disorder, dubbed "arterial calcification due to CD73 deficiency". Researchers analysed the DNA of five affected siblings and found that they all had mutations of the *NT5E* gene, which codes for the CD73 enzyme that produces adenosine - a molecule that helps prevent arteries from calcifying (*The New England Journal of Medicine*, vol 364, p 432). The findings offer targets for a treatment.

The discovery is impressive for its speed and technical prowess, says William Gahl, director of the UDP. "The role of adenosine was not known before."

Bloomberg Businessweek HealthDay

Executive Health February 02, 2011, 17:00 EST

Scientists Discover Rare Vascular Disease

DNA analysis identifies ACDC, where calcification occurs in arteries of legs, feet

WEDNESDAY, Feb. 2 (HealthDay News) -- Researchers report that DNA analysis has led to the identification of a new and extremely rare vascular disease, along with its genetic cause.

As described in the Feb. 3 issue of the *New England Journal of Medicine*, the debilitating, painful and progressive disease is defined by the calcification of arteries in both the hands and feet of patients, and in the vascular network below the waist. Arteries of the heart remain unaffected.

Labeling it "ACDC," the U.S. National Institutes of Health (NIH) research team has linked the disease's tell-tale calcium build-up to a deficiency in a protein called CD73. And while the disorder bears some symptoms in common with rheumatoid arthritis (such as leg and joint pain), the study authors stress that it is a wholly new disease.

"This is the first novel disease discovery identified through the collaborative and interdisciplinary approach employed by clinical researchers in the NIH Undiagnosed Diseases Program," NIH director Dr. Francis S. Collins said in an NIH news release. "This disorder previously baffled the medical field and evaded diagnosis when conventional methods were used."

To date, ACDC has been diagnosed in exactly nine living patients from three unrelated families, among whom the disorder has been linked to a mutation in the so-called "NT5E" gene. Five of the patients are siblings from a single family, and all five bore the NT5E mutation.

Over the past 100 years, seven additional cases bearing similarities to the nine cases currently cited have been reported, but none of those led to a clear diagnosis of the newly identified condition.

The nine patients in the current study sought care for pain and cramping in their calves, thighs, buttocks and feet. Calcium build-up in arterial walls was subsequently uncovered.

Surgery to redirect blood flow to different arterial passages was conducted in one case, along with amputation of the foot.

The researchers expressed hope that, having pinpointed the genetic roots of the disease, they can now develop better treatments for such patients.

-- Alan Mozes

SOURCE: U.S. National Institutes of Health, news release, Feb. 2, 2011

Arterial and Joint Calcifications May Have Genetic Basis

Last Updated: February 03, 2011.

Researchers may have identified a genetic basis for symptomatic arterial and joint calcifications, according to research published in the Feb. 3 issue of the *New England Journal of Medicine*.

***NT5E* mutations found in affected members of three families**

THURSDAY, Feb. 3 (HealthDay News) -- Researchers may have identified a genetic basis for symptomatic arterial and joint calcifications, according to research published in the Feb. 3 issue of the *New England Journal of Medicine*.

Cynthia St. Hilaire, Ph.D., of the National Institutes of Health in Bethesda, Md., and colleagues performed clinical, radiographic, and genetic studies of members of three families with symptomatic arterial calcifications to study the genetic basis of arterial classifications.

The researchers found that nine individuals had arterial calcifications; five were siblings in one family, three were siblings in the second, and one patient was identified from the third family. All three families had *NT5E* mutations that resulted in nonfunctional CD73. Cultured fibroblasts from affected members of the first family had substantially reduced expression of *NT5E* messenger RNA, CD73 protein, and enzyme activity, as well as increased alkaline phosphatase levels and accumulated calcium phosphate crystals. The researchers found that genetic rescue experiments normalized the CD73 and alkaline phosphatase activity in affected family members' cells and that adenosine treatment decreased the levels of alkaline phosphatase and calcification.

"We identified mutations in *NT5E* in members of three families with symptomatic arterial and joint calcifications. This gene encodes CD73, which converts AMP to adenosine, supporting a role for this metabolic pathway in inhibiting ectopic tissue calcification," the authors write.

Several authors are listed as inventors on a patent for the use of adenosine agonists to prevent arterial vascular calcification disorder.

SCRIP Newly launched SCRIP CLINICAL RESEARCH

US NIH scientists discover ACDC, exploring bisphosphonates as treatment

Today
Donna Young

After 18 months endeavour, scientists at the US National Institutes of Health (NIH) have discovered ACDC. No, not the Australian "Highway to Hell" rock band, founded in the 1970s by the brothers Young, Angus and Malcolm. Nor does the finding involve alternating current/direct current, or electricity in any way. Instead, the NIH's breakthrough entails a rare genetic disease, which the agency has dubbed arterial calcification due to CD73 deficiency – ACDC – whose cause had previously not been identified.

The discovery could lead to new therapies to treat the condition, with potential broader applications, NIH officials said.

The identification of the disease came about after a Mount Vernon, Kentucky physician, Dr Karen Saylor, had submitted a medical referral in May 2009 for two sisters with unexplained calcium buildup in their arteries to the NIH's Undiagnosed Diseases Programme (UDP). This programme examines conditions that have perplexed and eluded medical diagnoses, with enrolled patients undergoing extensive testing and expert consultations at no charge at the agency's world-renowned centre in Bethesda, Maryland.

ACDC is the first disease identified through the collaborative and interdisciplinary approach employed by the three-year old UDP programme, said Dr William Gahl, the programme's chief and clinical director of the NIH's National Human Genome Research Institute.

The two Kentucky women's x-rays showed a clear build up of calcium in the large vessels of their hips, legs and hands, he explained during a 1 February media briefing, held in advance of the 3 February *New England Journal of Medicine* article describing the finding.

"Their radiological images astounded us," Dr Gahl said.

The patients, Louise Benge, 56, and Paula Allen, 51, and two of their siblings had endured joint pain and cramping in the calves, thighs, buttocks and feet due to poor circulation for much of their lives.

Ms Benge first experienced unexplained leg pain in her 20s, she told reporters. While her doctors were able to determine that there was a calcium blockage in her blood vessels, they were baffled by the cause. Her younger sister, Ms Allen, first started seeing physicians in her late teens for arthritis-like symptoms in her hands, which later spread to her legs. Her doctors, who also were stumped by the cause of the condition, offered Ms Allen vascular surgery, but advised that the procedure would need to be repeated after five years, so she declined that therapy option, she said.

With the help of academic collaborators in San Francisco, London and Italy, the NIH eventually located a total of nine patients from three unrelated families to study the condition – one of whom had undergone vascular surgeries to reroute blood flow through alternate vessels and a joint amputation in the foot.

After conducting genetic analyses on the nine patients, the NIH researchers pinpointed the cause of the condition as mutations in the *NT5E* gene, which makes the CD73 protein, an enzyme that produces adenosine, a small molecule that protects the arteries from calcifying.

The NIH investigators performed tests to characterise the molecular basis of the arterial calcification disorder and to validate various molecular activities in cells with *NT5E* variants, and with that, they were able to show that elevated activity of tissue-nonspecific alkaline phosphatase (TNAP) was due to the lack of extracellular adenosine.

TNAP degrades an inhibitor of calcification, called pyrophosphate. The researchers therefore tied the elevation in TNAP activity with increases in arterial calcification.

They also suggested that the location of calcification may correspond to the distribution of specific adenosine receptors in the body.

With adenosine and alkaline phosphatase activity identified through the use of the cell-system models, the NIH is pursuing a therapeutic option, said Dr Manfred Boehm, an investigator in the NIH's National Heart, Lung and Blood Institute.

Certain compounds have been shown to be effective in other diseases that are related to ACDC, such as generalised arterial calcification of infancy, or GACI, which is caused by a mutation in the ectonucleotide pyrophosphatase–phosphodiesterase 1 (ENPP1) gene, which leads to decreased pyrophosphate levels, causing early-onset vascular calcification, myocardial infarction and often death in infancy, he explained.

The most notable drugs that have successfully been used to treat ENPP1 deficiency are bisphosphonates, he said. Based on those successes, the NIH researchers have hypothesised that those drugs may therefore potentially regulate calcium accumulation in the arteries, Dr Boehm said.

He noted that the agency currently is in the process of developing the protocol for a study of bisphosphonates in ACDC.

Because of their convenience, the NIH likely will use oral bisphosphonates in the trial, Dr Boehm said.

The NIH should know by early summer if it can move forward with the ACDC trial of bisphosphonates, Dr Boehm said, noting that the protocol first must go through the standard regulatory hurdles.

While bisphosphonates currently are the planned treatment compounds for the study, Dr Boehm said the agency also would continue to explore other options preclinically "to find the best drug for the patients".

Mutations Linked to Calcification in Arteries

By Nancy Walsh, Staff Writer, MedPage Today
February 02, 2011

MedPage Today Action Points

- Explain that genetic analysis of three families with lower extremity and joint calcification found mutations in the *NT5E* gene encoding an enzyme converting AMP to adenosine.

- Note that the deficiency in this metabolic pathway suggests a genetic basis for the disorder and possible treatment options such as bisphosphonates, which provide an "adenosine rescue."

Review

Mutations in the *NT5E* gene have been identified as the cause of symptomatic lower extremity arterial calcifications in three families, researchers reported.

Nine patients in these families have now been found to have an adult-onset disorder that results from missense and nonsense mutations in *NT5E*, according to William A. Gahl, MD, PhD, of the National Human Genome Research Institute in Bethesda, Md., and colleagues.

This gene encodes the enzyme CD73, which is involved in a metabolic pathway by which AMP is converted to adenosine on the surface of cells, the researchers explained in the Feb. 3 *New England Journal of Medicine.*

One family that included five siblings with intermittent claudication and abnormal ankle-brachial blood pressure index values was enrolled in the National Institutes of Health (NIH) Undiagnosed Diseases Program for assessment.

The proband in this family was a 54-year-old English woman whose parents were third cousins.

Radiography showed calcification with arteriomegaly in the legs, plus calcifications of the joint capsules in the ankles, feet, wrists, and fingers. Magnetic resonance angiography confirmed occlusion of several lower extremity arteries.

Her siblings also had femoropopliteal occlusion with arteriomegaly and remodeling, plus calcified obstructive lesions.

Genetic analysis identified a 22.4 Mb region on chromosome 6q14, homozygous in all the siblings but heterozygous in the parents, that contained 92 genes.

Three of these genes were known to be involved in degenerative calcification, and direct sequencing found a nonsense mutation, C.662C→A, in exon 3.

In the second family, the proband was a 68-year-old woman from northern Italy with possible consanguinous marriage in an earlier generation.

She had experienced unremitting pain in the joints of her hands for many years as an adolescent and young adult. Calcifications found in her legs had originally been diagnosed as chondrocalcinosis.

Two of her sisters, both in their seventies, had similar calcifications and pain in the lower extremities.

In this family, the mutation was found to be a missense mutation, c.1073G→A, in exon 5.

The proband in the third family was a 44-year-old woman who had been born to a French mother and an English father.

Diagnostic tests for lower extremity paresthesias revealed distal artery calcifications, without involvement of the coronary or carotid arteries. She had undergone a femoral-popliteal bypass one year earlier.

Analysis determined that this patient was a compound heterozygote for the same nonsense mutation found in the first family that resulted in a premature stop codon in exon 9.

Molecular and cellular studies revealed nearly absent expression of the CD73 protein, along with high levels of tissue-nonspecific alkaline phosphatase (TNAP), which is a crucial enzyme involved in calcification.

Only one previous inherited disorder of vascular calcification has previously been identified, which is a mendelian abnormality associated with a mutation in *ENPP1* that results in inadequate levels of pyrophosphate, causing vascular calcification, myocardial infarction, and usually death in childhood.

"In our adult patients, CD73 deficiency may not lead directly to decreased pyrophosphate levels, but the consequent reduction in extracellular adenosine levels apparently enhances TNAP activity," the researchers explained.

"We hypothesize that increased TNAP activity reduces pyrophosphate levels leading to calcification," they wrote.

Understanding of the genetic defect in these patients suggests the possibility of

corrective interventions, such as with bisphosphonates, according to Gahl and colleagues.

These drugs inhibit tissue calcification, are analogs of pyrophosphate, and have been used for the childhood-onset *ENPP1* deficiency.

In addition, according to the researchers, adenosine-receptor agonists or TNAP inhibitors such as lansoprazole (Prevacid) also could be investigated, initially in cell cultures and then in an animal model, if a suitable one can be developed.

This work was supported by the National Institutes of Health.

All authors declared no conflicts of interest.

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St. Hilaire C, et al "NT5E mutations and arterial calcifications" *N Engl J Med* 2011; 364: 432-442.

THE MEDICAL NEWS
from News-Medical.Net - Latest Medical News and Research from Around the World

Rare new disease identified – calcium accumulates in blood vessels

3. February 2011 17:26 Dr Ananya Mandal, MD

A new rare disease has been identified. Louise Benge's started having difficulty in walking when she was 25. She developed hardening and pain over her calves and hands. She worsened over the next 20 years. Her family physician in Mount Vernon, Ky., was at a loss, as were a vascular specialist, a hand specialist and a kidney specialist. Her two sisters and two brothers had the problem too, but no doctor could understand why.

Her X-rays showed that the blood vessels in her legs, feet and hands were accumulating calcium deposits like the scales that can form inside water pipes. The deposits had grown so thick that blood could hardly pass through. But calcium was only in the blood vessels of her legs and hands; her heart's vessels were spared, so it was not life threatening. She was given weekly infusions of a drug, sodium thiosulfate. This did not work.

Then at their wits' ends the doctors treating her sent her medical history to the Undiagnosed Diseases Program at the National Institutes of Health. The program, set up in 2008, relies on teams of specialists who use the most advanced tools of medicine and genomics to try to figure out the causes of diseases that have baffled doctors. The idea was that understanding rare diseases can give insights into more common ones, said Dr. William A. Gahl, director of the program.

Dr. Gahl said, "Patients who have rare diseases are often abandoned by the medical community…We don't know how to treat if we don't have a diagnosis. The way our society treats abandoned individuals is a measure of our society. It speaks to how our society treats the poorest among us." Ms Benge and her siblings were diagnosed with the new disease and their case is reported in the latest issue of *The New England Journal of Medicine*. It is caused by a mutation in a gene that prevents calcium from depositing in blood vessels.

The UDP took up the case on May 11, 2009, when Ms. Benge was 56, and her sister Paula Allen was 51. Nine people are suffering from the disease that is called "arterial calcification due to CD73 deficiency". Researchers analyzed the DNA of five affected siblings and found that they all had mutations of the *NT5E* gene, which codes for the CD73 enzyme that produces adenosine - a molecule that helps prevent arteries from calcifying.

The researchers found that cells use the gene to make extracellular adenosine, a common compound that in this case was needed to suppress calcification. No one had known about this metabolic pathway, said Dr. Manfred Boehm, a vascular biologist at the National Heart, Lung and Blood Institute. Dr. Dwight Towler, a bone endocrinologist at Washington University in St. Louis who was not part of the study also commended the study.

The investigators are putting together plans to test bisphosphonates (drugs used against osteoporosis) and submitting them to ethics boards for approval. "We hope to know in three or four months whether we can go forward," Dr. Gahl said.

NIH director Dr. Francis S. Collins said in an NIH news release, "This is the first novel disease discovery identified through the collaborative and interdisciplinary approach employed by clinical researchers in the NIH Undiagnosed Diseases Program… This disorder previously baffled the medical field and evaded diagnosis when conventional methods were used."

Diabetic Foot Blog - Southern Arizona Limb Salvage Alliance

Dedicated to amputation prevention and healing in persons with diabetes. For information on diabetes, diabetic foot complications, and the philosophy of caring for people with the disease.

Thursday, February 3, 2011

Vascular calcification: should we feed our patients prevacid?

This, thanks to our SALSA family member Tim Fisher of the United Arab Emirates, who identified this story from today's New England Journal of Medicine via MedPage Today. Although these were extreme cases of familial calcification, perhaps tissue nonspecific alkaline phosphatase (TNAP) inhibitors (like lansoprazole/prevacid) might be in our future!

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New gene mutations found involved in arterial calcification

FEBRUARY 3, 2011 | Sue Hughes

Bethesda, MD - New gene mutations have been discovered that are thought to be responsible for a rare disease causing extensive calcification of lower-extremity arteries and hand and foot joint capsules [1].

The researchers say their findings provide a new concept in vascular biology and suggest that adenosine plays a critical role in preventing calcification. While the calcification in the patients involved in this study appears to be different from that involved in coronary artery disease, the mechanisms involved may provide new leads to follow in coronary atherosclerosis research, they add.

The current study, reported in the February 3, 2011 issue of the *New England Journal of Medicine*, was conducted by a team led by **Dr Cynthia St Hilaire** (National Institutes of Health, Bethesda, MD).

Coauthor **Dr William Gahl** (National Institutes of Health) explained to heartwire that the disease, known as arterial calcification due to deficiency of CD73 (ACDC), is characterized by significant calcification of the tibial artery in the ankle and the femoral and popliteal arteries in the leg. The arteries lose all their elasticity, and the patient experiences claudication and pain in the legs. The calcification differs from that in coronary artery disease in that it forms mainly inside the vessel wall (media) rather than in the vessel intima.

For the current study, the researchers identified nine people with this condition from three different families. One of the patients also had calcification of the external carotid artery. Genetic studies showed that these patients had one of three mutations in the *NT5E* gene, which codes for the CD73 protein, which converts AMP to adenosine on the surface of various types of cells.

Inhibiting the inhibitors of calcification

The authors note that calcification is increasingly viewed as arising from a default biochemical pathway and that constant stimulation of inhibitory systems is required to prevent its occurrence. Levels of calcification depend critically on levels of pyrophosphate, a strong inhibitor of calcification, and tissue-nonspecific alkaline phosphatase (TNAP), which degrades pyrophosphate.

They believe that CD73 deficiency causes a reduction in extracellular adenosine, which enhances TNAP activity, which in turn reduces pyrophosphate levels, leading to calcification. They add that the selective involvement of lower-extremity arteries may be related to the distribution of particular adenosine receptors in these tissues.

Cultured fibroblasts from affected members of one of the families showed markedly reduced expression of *NT5E* messenger RNA, CD73 protein, and enzyme activity, as well as increased alkaline phosphatase levels and accumulated calcium-phosphate crystals. Genetic rescue experiments normalized the CD73 and alkaline phosphatase activity in the patients' cells, and adenosine treatment reduced the levels of alkaline phosphatase and calcification.

Discovery of this mechanism may lead to possible treatment options such as adenosinelike agents or bisphosphonates, which are pyrophosphate analogs and potent inhibitors of tissue calcification. The antithrombotic drug **dipyridamole** may also be worth investigating, as it inhibits cellular reuptake of adenosine. Other therapeutic possibilities include a direct inhibitor of TNAP such as **lansoprazole**.