

LAST CHANCE CLINIC

Some diseases defy diagnosis. **Brendan Maher** meets two people who hope that the US National Institutes of Health can help.

Dunham Aurelius is eager to take his shirt off and show his scars. One, a centimetre wide and roughly 20 long runs up his lower back and is from the placement of a steel rod to straighten his spine at the age of 14. Two others, looking like bullet wounds, are above his left buttock. But it's not his scars, nor his barrel-chested physique that have earned him the nickname 'ultimate fighting champion'. His urologist bestowed that title because of the fact that since the age of 22, Aurelius has passed a dozen and a half kidney stones — many, he's proud to say, without assistance. Aurelius is 39, a sculptor and a former triathlete with curly blond locks and a surfer's drawl. His wife, Michelle Barry Aurelius, jokes that he's like a human oyster. But the stones he grows are no smoothened pearls. At the cinema in 2008, Aurelius stepped out to use the bathroom. When he returned, he handed her the four-millimetre wide 'barnacle' of calcium phosphate his body had just expelled. She had noticed he was quiet that evening.

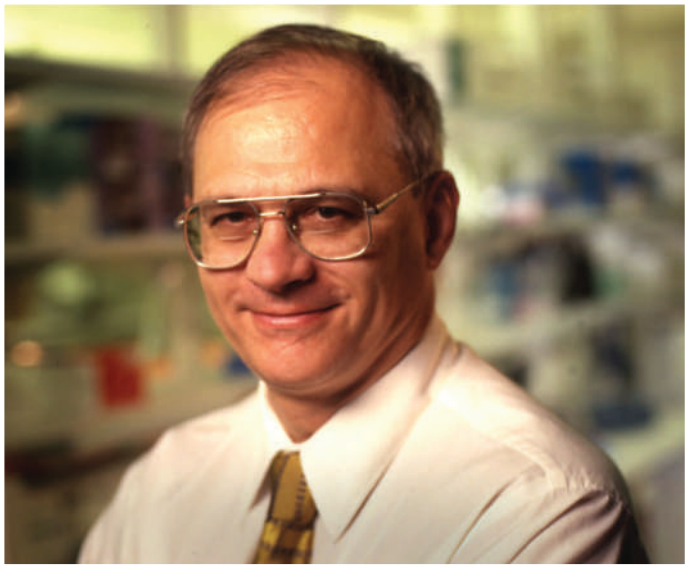
On a February morning this year, Aurelius and Barry are waiting in a hospital room in the sprawling Clinical Center on the campus of the US National Institutes of Health (NIH) in Bethesda, Maryland. They have travelled here from their home in Santa Fe, New Mexico, so that a small team of clinicians and research scientists can try to diagnose the mysterious disease that has dealt Aurelius more urological pain than most should have to bear. When William Gahl, the team's lead investigator and clinical director at the NIH's National Human Genome Research Institute (NHGRI) enters Aurelius' room at 9:50 a.m., he has a gaggle of clinical-genetics fellows in tow. He rattles through an introduction to Aurelius, and then stops himself. "Why don't you tell us," Gahl says, picking his words carefully, "why are you different from the average person?"

"There's nothing so complicated for a patient as not being able to put a name to their disease."
— Carl May

Elsewhere in the Clinical Center, another far-from-average person is awaiting time with Gahl. Sally Massagee, a 54-year-old certified public accountant from Hendersonville, North Carolina, is watching a neuromuscular specialist remove three deep-red slivers of muscle from her bicep. Although she was nervous going into the biopsy, Massagee jokes that she can spare the tissue. A little more than a decade ago, she started putting on weight. By the spring of 2007 she had gained nearly five kilograms on a compact 1.68-metre frame — all of it muscle. People around her thought she was training for competitive bodybuilding, but pain stopped her doing any exercise but tennis. Eventually she outgrew her clothes.

Massagee and Aurelius have few, if any, symptoms in common. What they do both have is a spot in the NIH's Undiagnosed Diseases Program, an effort to identify and characterize previously unknown diseases by drawing on the institution's 6,000 clinical and biomedical experts and the medical technologies at their fingertips. Gahl, a medical geneticist specializing in metabolic disorders, started the programme in May 2008 with $280,000 in pilot funding from the NIH's Office of Rare Diseases. It received $1.9 million more in its first year, and has been approved as a fully fledged NIH programme at $3.5 million per year for the next five. Patients such as Aurelius and Massagee (pictured above) hope that this financial and academic wealth can finally provide the diagnosis that has eluded all the other specialists they have consulted over the years. "There's nothing so complicated for a patient as not being able to put a name to their disease," says Carl May, a professor of medical sociology at Newcastle University, UK, who has studied doctor–patient relationships in chronic disease. "If we can't put a name to it, it's hard for others to see or understand, and most importantly to believe, that something is legitimate or warranted."

M. BARRY AURELIUS; NIH; S. R. MASSAGEE



E. BRANSON/NIH

L. SPILLERS

Team leader William Gahl and nurse practitioner Colleen Wahl work on finding new diagnoses.

The researchers want a diagnosis, too — but their motives are somewhat different. For them, Aurelius, Massagee and the other individuals are also a research opportunity, the chance to discover a new disease and potentially one that can be characterized at a genetic level. This could provide a new foothold in understanding human biology and perhaps the origins of other, more common diseases. Such diagnoses can result in high-profile publications and spur the development of new fields. As Clemens Bergwitz at the Massachusetts General Hospital in Cambridge puts it, the programme allows scientists to "make use of the human mutation pool".

That approach is not new: throughout much of medical history clinicians with just the right background have stumbled on just the right patients to come up with a new diagnosis. Gahl says that one way of thinking about the Undiagnosed Diseases Program "is to reduce that need for serendipity" by setting out to find the unusual cases and throw at them everything research has to offer, including individualized sequencing of candidate genes and a genome-wide scan of genetic variations. "The sort of modern twist to this classic approach is the molecular-biology techniques available," says NIH endocrinologist Michael Collins, who has been working on Aurelius's case.

Aurelius and Massagee spotlight the relationship between subject and scientist at its most focused, modern and expensive. The question is: what, if anything, will each side gain?

Stony symptoms

Aurelius reclines in a hospital bed while Gahl runs through his medical history. The stones are generally calcium phosphate. The largest two-to-three-centimetre stone was removed by surgery, which resulted in a perforated colon and left Aurelius with the bullet-hole scars in his back. He has regular gastrointestinal discomfort, and Aurelius says that he has very high calcium and vitamin D levels in his blood. Gahl asks how calcium in food affects him. "I avoid it in most cases. It makes me feel distended. If I had a bowl of ice cream I'd be miserable."

"Ever take a vitamin D pill?" Gahl asks.

"No, but we could try it!" says Aurelius. Like others involved in the programme, Aurelius is happy to be a part of it even though he knows the chances of a diagnosis, let alone a treatment, are low. It means he hasn't been given up on. "Most doctors would throw their hands up," Aurelius says, describing his quest over the years. Gahl, no enemy to truth in levity, replies: "We may too, but we'll do it behind closed doors, after you're gone."

After Aurelius is sent off for a bone-density scan and an ultrasound on his kidneys, the closed-door discussion begins. Gahl meets with a group of experts working on the case: Collins, nurse practitioner Colleen Wahl, pathologist Panagiota Andreopoulou, and attending genetics fellow Galina Nesterova. The meeting moves quickly as they bandy about the names of genes that might be responsible for Aurelius's unusual blood test results.

At first the talk centres on a protein called fibroblast growth factor 23 (FGF23), which lowers phosphorus absorption and, through a series of regulatory loops, helps to cap the production of active vitamin D. Too much vitamin D can result in high calcium levels in the blood and urine — hypercalciuria — which leads to kidney stones. Nesterova, who worked as a nephrologist in Russia before coming to the United States, has another idea. She suggests looking at two genes, *CYP27B1* and *CYP24A1*, that activate and deactivate vitamin D, respectively. Her hunch is based in part on some ongoing work with two sisters with elevated vitamin D levels and highly calcified kidneys, who she also suspects of having mutations in these or related genes.

Collins is sceptical, not wanting to close the door on alternative explanations. Aurelius has low blood phosphorus levels, and Collins wonders whether the underlying problem could lie in the kidneys: if they are excreting too much phosphorus, this would feed back to the body, instructing it to manufacture more active vitamin D. He posits a mutation in one of the genes for a sodium/phosphate transporter, called *SLC34A3*, in the kidney tubule wall that may be causing Aurelius to dump out phosphorous.

"It's neat that in-house, we've got Mike," says Gahl later, referring to Collins. "He knows 100 times as much about this as me." Collins was first introduced to Aurelius's case through a monthly meeting for the Undiagnosed Diseases Program in which upwards of 50 basic- and clinical-research scientists sit and listen to presentations on potential patients to decide who should be invited into the programme. During the screening process they try to pull out diseases with simple genetic roots, ones probably caused by a single mutated gene, or a deletion or duplication of a large chunk of DNA. Maybe there is a mention of a family history in a chart, or signs that the symptoms are related to an organ system in a way that suggests a single unifying cause. Complex disorders

NIH

Experts gather for monthly meetings at the NIH Clinical Center (left) to discuss which patients to admit.

NIH

with roots in multiple genes have to take a back seat, says Gahl: "This is triage, and triage means you go after what you can do." Since its inception, the programme has received more than 2,100 inquiries from doctors around the world, reviewed more than 900 full applications and, so far, seen little more than half of the 160 patients that, like Massagee and Aurelius, have been invited to come for an intensive week of tests and consultations.

As Nesterova and Collins spar collegially, Gahl finds a possible way to settle the debate. "Let's get the FGF23 back and have a plan then," he says, referring to a test already requested that will measure the amount of the hormone in Aurelius's blood. A high concentration might change the types of genes that they would test, probably striking *SLC34A3* from the list.

Less than an hour after talking vitamin D metabolism, Gahl is discussing muscle physiology and genetic tests he hopes to run on Massagee's blood. Like Aurelius, Massagee has already been through three and a half days of testing and has just a few more appointments to go. One of these is a wrap-up interview with Gahl and a different team of specialists, including Justin Kwan, the doctor who did the muscle biopsy, and clinician Irini Manoli from the NHGRI.

Accompanied by her husband, Massagee is dressed in velour tracksuit pants and an oversized man's dress shirt. She looks fatigued, but alert, her brown eyes peeking out over her puffy, muscle-tightened cheeks. When in 2008 doctors at Duke University Medical Center in Durham, North Carolina, did a magnetic resonance imaging test to try to diagnose her condition, they were shocked to see that even the orbital muscles that control her eye movements seemed to have doubled in size. Massagee says that when she had been exercising, the muscles had tone, and she believes many of the specialists she saw suspected she was taking steroids. Now the muscles are rock-hard, painful and toneless, weighing her down and leaving her exhausted.

Massagee's case has also drawn in experts from across the NIH, including Alexandra McPherron of the National Institute of Diabetes and Digestive and Kidney Diseases. For her PhD thesis, McPherron characterized a protein that doubled the skeletal muscle of mice when it was mutated (A. C. McPherron, A. M. Lawler and S.-J. Lee *Nature* **387,** 83–90; 1997) and she's worked on it ever since. Later named myostatin, the protein was found to be the molecular culprit behind heavily muscled cattle, sheep, dogs and in one reported case of a human — a baby boy born in Germany with massive muscles in his thighs and upper arms (M. Schuelke *et al. N. Engl. J. Med.* **350,** 2682–2688; 2004). So, when a muscle-laden woman was accepted to the Undiagnosed Diseases Program, myostatin — and McPherron — were on Gahl's mind. He sent over photos of Massagee and eventually went to visit McPherron in her office. "I've never met a patient before," McPherron says. But she agreed to get involved with this one.

"The physician in me is interested in helping people. The scientific part of us goes after the new disease areas."
— William Gahl

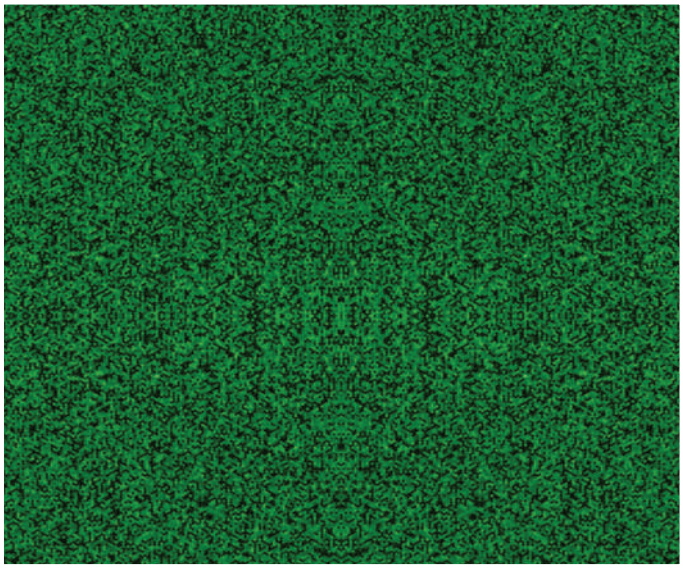
A question of need

Humans present experimental challenges that McPherron's mice do not. "The big question," she says, "was, do I want a muscle biopsy?" McPherron would need some tissue to do a thorough analysis of Massagee's myostatin expression levels. She knew the discomfort involved, but the only material already available was old and not prepared in a way that would allow protein extraction and RNA analysis. "I was hemming and hawing," she says, but when the rest of the team decided to go for it anyway, she decided she should be there to see Massagee and to carry the tissue back to the lab herself. The main results Gahl's team wanted would be coming from the biopsy tissue, both from McPherron's lab and from the laboratory at the Armed Forces Institute of Pathology (AFIP) in Washington DC that would be doing a full histopathological work-up.

There are other tests to run. Gahl explains to Massagee the purpose of a 'million SNP array', an assay that is used commonly in research genetics but rarely in clinical diagnostics. The array looks for single nucleotide polymorphisms (SNPs), spots in the genome that differ between individuals with relatively well known frequency. The SNPs serve as landmarks and if one that is expected to neighbour another is missing or doubled up, it can show where DNA has been deleted or duplicated, perhaps pointing to the genetic root of a disease. Gahl's team does an assay for everyone who enters the Undiagnosed Diseases Program, and often for their family members too.

Gahl appears to take a certain pleasure in explaining the method, but he seems to lose Massagee while trying to explain such concepts as 'loss of heterozygosity'. Still, he is patient with her, mirroring her wonder over how some tests work and assuring her that it is pointless for the time being to worry about what the results might mean for her children. "We're operating on best guesses and a lot of ignorance," he says.

M. BARRY AURELIUS



T. C. MARKELLO

The DNA of sculptor Dunham Aurelius was analysed with a 'million SNP' array.

But of course the patients do worry. Just days before Aurelius and his wife arrived in Bethesda, they found out that she was pregnant. Although extremely early in the pregnancy, they told the doctors in case there was anything they should know. The results of genetic tests take on greater significance when another generation could inherit the result. Aurelius explained the frustration of not knowing what to expect from his body or for his children. "Everything's good with me, but I have this alien disease. I keep wondering what's going to happen."

At the end of a week of tests, Massagee and Aurelius are discharged and return to their homes to await their results. "It's going to be nice to be able to walk out of here, if not with a diagnosis, with at least the next step," says Aurelius. Massagee effuses gratitude and says how wonderful everyone at the NIH has been.

"At its best it is a wonderful place," Gahl says, adding dryly, "at its worst it's a government organization."

Over the next few months, work starts on the data from Massagee and Aurelius. The test for the FGF23 levels in Aurelius's blood comes back normal. Nesterova had already begun to sequence *CYP27B1* and *CYP24A1*, and in May she finds that one copy of Aurelius's *CYP24A1* is missing three base pairs. This 'microdeletion' is not currently listed in any databases of known human variation, suggesting that it may be a novel change. It could be disabling or at least limiting Aurelius's ability to deactivate vitamin D, explaining his high levels of the vitamin. Then again, it could be harmless. "It's hard to put the weight of significance on these findings, for now," Nesterova says.

Collins still favours his hypothesis about the sodium/phosphate transporter, and at his urging Aurelius consents to send DNA samples to a programme, run by Bergwitz, that is sequencing genes that code for various versions of the transporter. Others with mutations in these genes have low phosphate levels and bone disorders such as rickets. "Our interest is to find more mutations," Bergwitz says, ones that create different symptoms. These could reveal what parts of the genes and their corresponding protein actually do, be it ion-pumping mechanics or insertion into cell membranes.

The million-SNP arrays come back with reams of data. Thomas Markello, who runs the studies for the programme, says that they spit out many hits but little in the way of answers. Raw data suggest that each person has between 3 and 10 positions in the genome in which both copies of a given genetic region are deleted, plus 50–200 instances each of single-copy gene deletions and duplications, any of which — or none of which — could be involved. "This is not what most physicians are used to seeing," Markello says. So far, the SNP arrays have helped with just one diagnosis. But this is a research programme, and part of the research is to determine how useful these techniques can be. Moreover, the data and samples, stored at the NHGRI, may still prove informative for future studies. Markello calls the programme "training wheels" for using whole-genome sequencing in the clinic, in which the number of genetic differences found in a given individual will go up many orders of magnitude but their clinical significance will be even harder to tease out. In the near future, he says, those in the programme will have their entire genome sequenced.

"It's going to be nice to be able to walk out of here, if not with a diagnosis, with at least the next step." — Dunham Aurelius

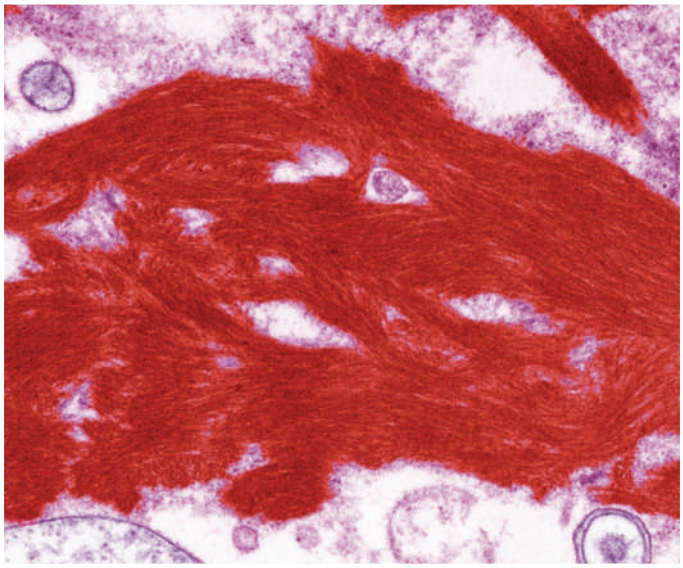
Hints of progress

On 8 April, Massagee e-mailed Manoli, her main contact point at the programme. Massagee was more fatigued than ever, having to stop what she was doing every 20 minutes and take a break. Work was becoming difficult, and she couldn't walk more than a block without losing her breath. Manoli called her back that day to ask if she could come back to Bethesda for more testing. Although the full report from the AFIP had not yet come back, the pathologists had found hints that proteins were building up in the walls of the blood vessels that feed Massagee's muscles.

On her second visit to the NIH, Massagee got her diagnosis. She had amyloid light-chain, or AL, amyloidosis, a rare disorder that is tied to the bone marrow's abnormal production of immune cells that make immunoglobulin proteins. Excess immunoglobulin accumulates into the proteinaceous build-ups that were lining some of her blood vessels.

Around 1,200 to 3,200 cases of AL amyloidosis are reported each year in the United States. Amyloid can build up in pretty much any tissue or organ, but Massagee's presentation in skeletal muscle is especially rare. The researchers do not know why the immunoglobulin caused her muscles to bulk up. But happily for Massagee, her heart muscles seemed to be unaffected and there was no serious damage to her kidneys, which can lead to death. Manoli contacted Morie Gertz, who studies the disease at the Mayo Clinic in Rochester, Minnesota, and pushed for a swift appointment. Gertz's team saw Massagee the following week to determine whether she would be a good candidate for a clinical trial

S. R. MASSAGEE



S. GSCHMEISSNER/SPL

Sally Massagee and her husband found out she had a type of amyloidosis, which causes protein build-up in tissues.

to treat the disease with chemotherapy and autologous bone-marrow transplantation.

She was. On 19 June, Massagee underwent the bone-marrow transplantation procedure. Afterwards she developed a condition called peri-engraftment syndrome, a poorly understood complication of autologous transplants that made her very sick. But now she says she's feeling stronger every day. Her doctors are uncertain whether treatment of the haematological condition will reverse the build up of muscle. But Massagee says that her muscles feel softer to the touch already, and that she considers the NIH programme to have saved her life.

Gahl's team was also pleased to get a diagnosis, even if it was one that is not new to medicine. None of their hunches about the involvement of myostatin came true. But McPherron is keeping Massagee's muscle sample in the freezer. She hopes to use it to investigate how the accumulation of immunoglobulin led to such an overgrowth of muscle. One idea is that the build up, or an inflammatory response to it, activated the satellite stem cells that normally divide to create new muscle tissue. But anything McPherron could do with it would be extremely preliminary.

"Our time will come with respect to new diseases," says Gahl. "We're very pleased to find different presentations of known disease, and I wouldn't discount the learning process." The team has made other such diagnoses. Gahl says that they have recognized a handful of cases of multiple sclerosis for patients enrolled in the programme and they were able to diagnose an atypical case of lymphoma simply from a chart review. But these are the happy endings. There are still upwards of 50 open cases in Gahl's files. May, the sociologist at Newcastle University, says that the doctors involved in the Undiagnosed Diseases Program are unusual in this sense because they know that the vast majority of their cases will never be solved. "There is a conflict there between having someone who is an interesting case and somebody who is going to be evidence of one's failure."

Gahl brings up one other statistic, and it's clear that it weighs heavily on him. Twelve of the patients who applied to the programme have died so far. One of these, a young woman called Summer Stiers, had serious symptoms affecting many of her organ systems and was the subject of several news stories earlier this year. The tests that Gahl's team ran when she was at the NIH generated few concrete leads. Stiers decided, with her local doctors' acquiescence, to discontinue the regular dialysis and other treatments that had been keeping her alive. She died within three days. Stiers had called Gahl a few days before this, in part to make final arrangements for her body to be shipped to the NIH for further study. Even at the end of her quest for a diagnosis, she wanted to help.

Aurelius, in Santa Fe, is still anxiously awaiting news. Nesterova contacted him in July to tell him that she wished to publish an abstract about the *CYP24A1* microdeletion for a meeting on metabolic disorders in San Diego, California, this month. She suspects that it is responsible for his symptoms; if it is, it could mean the identification of an entirely new genetic disease. Although excited by this possibility, Nesterova is reluctant to become too confident until she can do more follow-up work to show definitively that the mutation affects the function or levels of the protein it encodes. Her colleagues have been sequencing the gene in 100 healthy controls to see whether the deletion is simply a harmless variant. "She's cautious and thorough," Aurelius says.

Despite being happy that someone is still working on his case, Aurelius says the pace still feels slow. "At the end of the day what I want to stop is the kidney stones because I don't want to have renal failure," he says. Another stone is currently growing in his left kidney. Aurelius and his wife are preparing for the birth of their child in late October, and he continues with his sculptures — large, craggy, organic-looking pieces in bronze and wood that unmistakably evoke the calcium phosphate stones that have caused him so much pain. He is even planning a show for the Clinical Center starting in November. He's promised that a portion of the proceeds from any sales will go to a patients' fund at the NIH. "It's rare in life when you feel like someone gives so much to you — taking me out there for a week, getting all these doctors together. It's important to give back to that."

And Gahl, like the other doctors in the programme, continues to wrestle with his dual motivations for it. "The physician in me is interested in helping people," he says. "The scientific part of us goes after the new disease areas. That's very stimulating — to be the first to discover something. I think all of us feel that way". Even if they don't find either diagnosis or new disease, the Undiagnosed Diseases Program offers at least an extension of hope for those who enter it.

"I'm astounded at how appreciative they are of our failed efforts," says Gahl. ■

Brendan Maher is *Nature*'s biology features editor.

SCIENCEPHOTOS/ALAMY

EVIDENCE FOR MONOPOLES
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World climate services framework agreed

GENEVA

A global framework to supply on-demand climate predictions to governments, businesses and individuals is moving closer to reality.

On 3 September, delegates representing 155 nations at the World Climate Conference in Geneva, Switzerland, agreed that a body should be established to supply such 'climate services' to users ranging from national governments to individual farmers. The service would be particularly helpful for developing nations, many of which lack access to the weather and climate observations needed to plan their strategies for adapting to climate change.

Over the next four months, an independent task force set up by the World Meteorological Organization will work out how to make this vision a reality. A 12-month consultation process with signatory nations will follow.

"It's about time we got serious," says climatologist Jonathan Overpeck of the University of Arizona in Tucson. "We can save wealth and properties if we get climate information into the hands of decision-makers."

But a global climate service will face a host of scientific and political hurdles. Negotiating data collection and sharing among member states will be a big challenge, for example. Some countries are already baulking at the suggestion that they will need to supply the service with data, citing issues such as national security or commercial interests that would prevent disclosure. In response, Martin Visbeck of the Leibniz Institute of Marine Sciences at the University of Kiel in Germany says that one option would be to allow "data of convenience tailored for specific purposes [to] be commercialized", while allowing "fundamental information to be freely available".

Climate scientists will also have to improve the quality of the climate projections that the service could provide. Today's global climate models predict how climate variables, such as temperature and rainfall, will change over the coming century at scales of several hundred kilometres. But scientists are hopeful that with further research they could bring that down to just tens of kilometres, covering timescales of a decade or less.

In the meantime, individual nations are forging ahead with their own climate-services centres. In July, Germany opened a centre in Hamburg, and the United States is discussing a national climate service. ■

Olive Heffernan

For a longer version of this story, see http://tinyurl.com/climate-service.

Correction
The News Feature 'Last chance clinic' (*Nature* **460,** 1071–1075; 2009) inadvertently located Massachusetts General Hospital in Cambridge. It is in Boston.