# The Angelman Approach: Hacking DNA to Treat a Rare Disease

by

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ABSTRACT

One of every hundred children is born with a disease caused by a single abnormal gene. In the case of Angelman Syndrome, the genetic defect leaves patients mentally disabled, largely or completely unable to speak, and prone to seizures and sleep difficulties. Many Angelman researchers are trying to figure out precisely how those symptoms develop, but why study all the individual effects when you could go right to the root of the problem? Recent advances in medicine and technology are increasingly allowing clinicians to treat genetic illnesses by directly manipulating patients' DNA, and a number of scientists are now investigating ways to leverage those discoveries for individuals with Angelman Syndrome. Their work could lead to potent therapies for the disease, and – maybe – even a cure.

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Allyson Berent was instantly recognizable from the photo posted online by the Foundation for Angelman Syndrome Therapeutics (FAST). My first meeting with the young, lanky, New York veterinarian who serves as FAST's Chief Scientific Officer took place in the coffee shop on the first floor of Chicago's Hyatt Regency Hotel. But barely an instant after shaking her hand, our introduction was interrupted by a squeal of delight erupting from behind us. A short, bespectacled woman with salt-and-pepper hair dashed over, wrapped Berent in an enthusiastic embrace, and burst into tears.

This moment was also Donna Young's first time greeting Berent in person. Affectionately known as "spamming nana" due to her zealous activity on social media, Young had only communicated with Berent on Facebook about Young's 14-year-old granddaughter with Angelman Syndrome, the genetic disorder responsible for the "AS" in "FAST." Since 2008, FAST has worked tirelessly to raise money and stimulate research into treatments for this rare neurological illness. Berent is a key part of that mission, and seeing her in-person for the first time overwhelmed the passionate grandmother. "I am forever grateful to her," Young would later say.* "I adored her from afar and finally met her face-to-face."

Angelman Syndrome afflicts approximately one in every 12,000 to 20,000 individuals, according to the National Organization for Rare Disorders. The disease causes a wide array of symptoms including cognitive disability; delays in hitting major developmental milestones like walking; difficulties with balance and movement; seizures; sleep problems; and a severely limited – and often complete – inability to speak. Intriguingly, individuals with the condition – called "Angels" in the Angelman community – possess a sunny disposition and outgoing personality. This trait, in combination with patients' typically jerky and uncoordinated movements, led the man who first described the illness, Harry Angelman, to (somewhat pejoratively) call it "happy puppet syndrome."

Young was among nearly a thousand parents, scientists, and others who converged in the windy city in early December 2016 for the 5th annual FAST Global Summit on Angelman Syndrome, a two-day event organized by Berent and her FAST colleagues to bring together the Angelman community and inform them about the latest research. The event concludes each year with the FAST Gala, a major fundraiser that many Angelman parents look forward to all year.

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* In a Facebook message

And this time, there was even more excitement than usual. A mere eight years after FAST's inception, the Angelman community believes the outlook for those with AS looks very promising. Pharmaceutical company Ovid Therapeutics will soon begin human trials of a drug, called Gaboxadol, that could ease Angels' seizures and movement problems and potentially even improve their cognitive abilities. But perhaps even more exciting is the research being done by two other pharmaceutical companies, Agilis Biotherapeutics and Ionis Pharmaceuticals. Each company is working towards clinical trials for therapies aimed at correcting the genetic defects that cause AS. Unlike Gaboxadol, which targets a specific system that is abnormal in Angels' brains, these genetic treatments may do more than relieve a few symptoms. Berent and Young hope that, within a decade, they could get the one thing they have always wanted for their loved ones: a cure for Angelman Syndrome.

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Even before joining FAST in 2016, Berent was no stranger to either Angelman Syndrome or clinical trials. In addition to her role at FAST, Berent serves as the Director of Interventional Endoscopy at New York City's Animal Medical Center, one of the world's largest animal hospitals. She has over a decade of experience in creating and testing innovative treatments for dogs and cats. But Berent also has a two-year-old daughter named Quincy who was diagnosed with AS on November 19, 2014, at the age of seven months.

Most children with AS are diagnosed around the two-year mark, but because of Berent's medical background and experience with her healthy first daughter, she quickly noticed Quincy's developmental delays. Others, if their angel is their first child, often don't have any context for gauging their baby's slow development. "Because of that they keep being told by their pediatrician that it's just a small delay and they'll grow out of it," Berent said. As a result, according to her, most Angelman parents spend a year or more fighting with their child's physician over what, if anything, is wrong. Eventually, the children will experience a dramatic event like a seizure or their physician will notice that they haven't started walking or talking by age two, "and then somebody does a genetic test," she said.

Berent's clinical training also shaped her response to what she consistently refers to as "my diagnosis." "My first reaction was, 'What can I do? How can I help her?'" she said. Despite what doctors and even other Angelman advocates kept telling her, she discovered that several

experimental treatments had already substantially improved functioning in mouse models of the condition by directly altering their DNA. Prior to that, these mice had similar symptoms to those of human patients. "The more I read about this and the more I understood this, I also realized that we can fix this," she said. Yet, until Berent became involved with FAST, she was unaware of any efforts to move those gene-focused treatments from mice into humans. "Unbeknownst to me, all of these conversations were actually happening with FAST and these pharmaceutical companies, but not being made public until they made the final decision to go forward," Berent said. Upon joining FAST, it became Berent's responsibility to make such information public. "Parents need to have optimism," she continued. "They need to know that there's a chance."

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Though Dr. Angelman first described AS in 1965, little was known about its root cause until 1997, when Dr. Arthur Beaudet, a professor of molecular and cellular biology at Baylor College of Medicine in Houston, Texas, discovered that a gene called *UBE3A* is responsible for AS. This discovery, along with his lab's subsequent creation of the first mouse model of the condition, has led many to refer to Beaudet as "the godfather of Angelman Syndrome."

Nearly everyone inherits one copy of the *UBE3A* gene from each parent. But because the brain's neurons can function normally with only one copy, a process called imprinting renders the gene received from the father inactive in those cells at some unknown point during the growth of a baby's brain. Angelman Syndrome occurs when the remaining maternal gene is rendered unable to produce the molecule its code creates, leaving someone with no working copies of *UBE3A* in their neurons.

AS can emerge in several different ways, leading to some variation in the severity of patients' symptoms. The vast majority of Angels – roughly seventy to eighty percent – are "deletion-positive," meaning they are missing a specific, large segment of DNA that includes the maternal copy of *UBE3A* and several nearby genes. Another five to eleven percent have some other alteration in the mother's copy that similarly renders it nonfunctional , while about six percent have a defect in the gene's "imprinting center" that causes the maternal gene to be silenced along with the paternal copy. An estimated three to seven percent of patients have two copies of *UBE3A* from their father and none from their mother, a condition known as paternal uniparental disomy (UPD). The normal imprinting of these redundant paternal genes leaves these

children without any active copies of *UBE3A* in their brains. Finally, some patients appear to have none of those genetic issues despite displaying all the classic signs of AS. Symptoms tend to be most severe in deletion-positive individuals and less so in patients with UPD or the imprinting center defect.

Unlike many genetic disorders that affect the brain, which typically alter its structure, children with Angelman Syndrome develop normally in the womb and at birth have brains that look normal. However, the lack of a functioning *UBE3A* gene at some point causes widespread changes to the chemicals in their brains. Ovid's drug Gaboxadol works by partially correcting one of these changes, but while this could certainly have a great therapeutic effect, it's not a panacea. Genetic treatments for AS, on the other hand, could restore all of the systems thrown into chaos by the lack of an active *UBE3A* gene, even without researchers' knowing exactly which chemicals or processes are affected. Indeed, directly targeting the gene in mouse models of AS has been shown to improve the animals' motor coordination and performance on certain tests of learning and memory, sometimes to the point where the researchers couldn't tell the difference between the mouse models and genetically normal mice. "And this is in [adult mice], so this is like a 40-year-old person," Berent said. Consequently, restoring the gene's function in a days-old infant could have markedly greater effects. While it remains to be seen how the results from mice will translate into humans and how it might differently affect patients of various ages, Berent is optimistic about the approach's potential, especially for the youngest individuals. "As a newborn you're probably going to get a cure in all symptoms," she said.

Regardless of whether genetic approaches provide a complete cure for AS, the advent of gene therapy is set to fundamentally change the way genetic disorders are treated. "It's a pivotal time" for gene therapy in general, Berent said. "Angelman Syndrome is just really lucky that we're at the right place at the right time.

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"That's funny."

Dr. Edwin Weeber's ears pricked up when he heard those two words. A technician in his lab at Baylor, where Weeber was working as a postdoc, had spent nearly a year and a half staring at tiny colored bands on blocks of translucent gel. She had been searching for a particular kind of

molecule found in larger quantities in the brains of Angels – that is, if it existed at all. It had taken so long that the technician "thought I was crazy for doing all these [tests] to try to find this protein," Weeber said. So when he heard her remark, he rushed out of his office to take a look for himself.

"What's funny?" he asked her as she moved aside for him to examine the jiggling gel. What she had seen would fundamentally change the way researchers thought about Angelman Syndrome.

"This was a huge moment in science where looking at one piece of evidence, I knew something that nobody else in the world knew, and I knew the implications of it" said Weeber, who now runs his own lab at the University of Southern Florida. "It happens very few times in your career."

Weeber had long been curious how the lack of a working *UBE3A* gene exactly alters the brain. The protein, UBE3A[†], that the gene normally produces helps the brain get rid of old or malfunctioning molecules by attaching a certain chemical tag to them. Over time, a molecule will take on more and more of these tags, and the cell throws away ones that accrue too many of them.

UBE3A's role in this process of cellular housekeeping led Weeber to theorize that its absence may cause AS by permitting certain molecules to accumulate in the brain in the same way Alzheimer's disease is thought to result in part from the build-up of damaged proteins. For over a year, Weeber and his colleagues searched for any substance present at abnormal levels in the brains of Angels, but even after examining every chemical known to play a role in learning and memory, they found nothing. "So I came up with this crazy idea," Weeber said.

In a study of sea slugs, another research group had earlier discovered an alternative role for those tags. Instead of marking proteins for destruction, they influence a process called phosphorylation, which attaches a different label to certain molecules in the slugs' neurons; molecules that have undergone this procedure are described as "phosphorylated." The discovery of this system made Weeber wonder whether a lack of *UBE3A's* chemical tags might affect

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[†] Scientists traditionally italicize the names of genes but not the names of proteins, even when their names are the same, so the *UBE3A* gene produces a protein also called UBE3A, but not italicized.

phosphorylation in the Angelman brain in some way. As a result, his lab focused its search on compounds whose behavior changes when they are phosphorylated.

Within that test that caught the lab tech's attention, Weeber observed a protein, called CAMKII, whose phosphorylated form was more abundant in the brains of AS mice than those of typical mice. "That was the only thing biochemically that we had ever seen different in the AS brain," he said. Knowing that CAMKII plays a critical role in learning and memory, Weeber began collaborating with Dr. Ype Elgersma, an assistant professor of molecular neuroscience at Erasmus Medical Center in the Netherlands, to test what might happen to mice when their brains had too much phosphorylated CAMKII. Elgersma discovered that this change gave otherwise normal mice many of the problems – memory deficits, seizures, motor difficulties – that are seen in mouse models of AS. "By doing something completely different from UBE3A, we could mimic all the symptoms that we see in the AS mouse," Weeber said. Weeber and Elgersma ultimately explained this phenomenon by showing that phosphorylation reduces CAMKII's ability to do its job in brain cells.

Next, Weeber and Elgersma created a group of Angelman mice with modified *CAMKII* genes that produced a form of the compound that could *not* be phosphorylated. Unlike their parents, those mice had none of the symptoms of Angelman Syndrome. The two scientists concluded that mice need the *UBE3A* gene to curb the levels of phosphorylated CAMKII protein in their brains.

These results, published in 2007, provided the first proof that a comprehensive therapy for Angelman Syndrome was not just a pipe dream. Before this development, it was widely believed that Angels' neurological problems began while their brains were developing in the womb, limiting clinicians to merely curbing the conditions' later effects with intensive physical, occupational, and speech therapy, coupled with drugs that control similar symptoms like seizures in other disorders. But because the *CAMKII* gene does not become active until after birth, Weeber's discovery suggested doctors could do more than just treat the symptoms. "There's a lot of genetic disorders out there where the wiring of the brain, how the brain is formed, and how the neurons figure out where they're supposed to go – you disrupt that and you end up with a severe neurological disorder that you can't do anything about," Weeber said. "With Angelman, the brain developed normally. It wasn't a developmental problem; it was a biochemical problem."

In other words, the Angelman brain is like a car engine filled with molasses instead of gasoline – the engine would work fine if it just had the right material inside it. Just as drugs have been developed to readjust the brain chemistry of individuals with illnesses like schizophrenia and depression, therapeutics could theoretically be created to do the same thing in AS.

Unfortunately, Weeber said, targeting CAMKII to treat human Angels is not a very promising approach. Altering the *CAMKII* gene in humans in the same way he and Elgersma did in mice is not currently feasible and might not be all that helpful in any case. Despite the encouraging conclusions of his 2007 paper, Weeber now believes that CAMKII plays only a small role in AS. "I hate to go back and say one of the biggest papers I ever published was probably all malarkey, but we probably over-interpreted that data," Weeber said. But science often works like this – what looks like a dead end can lead to a new angle for research. In this case, while manipulating CAMKII specifically may not be useful, Weeber's experiments nevertheless spurred further attempts to correct the abnormal blend of chemicals in Angels' brains.

Fortunately, scientists may not need to know exactly how the lack of a working *UBE3A* gene in the brain impacts CAMKII or other molecules to cause Angelman Syndrome. The genetics behind AS are like an oil spill in the open ocean, causing an array of complex consequences that may never be fully understood. Rather than try to correct all these individual problems, the most effective strategy is just to clean up the oil – or, better yet, to prevent the spill in the first place. "We don't know the nitty gritty mechanisms of the gene," Berent said. But by giving Angels a normal copy of the gene, "you're probably going to fill all those holes and it doesn't matter."

Weeber's approach to giving Angels a working copy of the *UBE3A* gene rests on a procedure called gene therapy. The concept – first theorized in 1972 by Dr. Theodore Friedmann, a professor of pediatrics at the University of California, San Diego, and Dr. Richard Roblin of the Infection Disease Unit at Massachusetts General Hospital in Boston – basically boils down to replacing the faulty DNA responsible for genetic diseases with normal DNA from outside a person's own body. While there are several methods for accomplishing this, which scientists call "vectors," the most common is to use a virus that has had the desired DNA inserted into its genome. Natural viruses reproduce by injecting their DNA into their hosts' cells, turning

those cells into miniature factories that churn out copies of the virus, including all of the proteins encoded by the virus' genes. That's how Weeber hopes to replace the *UBE3A* gene in Angels: with a virus that has had all its harmful genes replaced with a healthy copy of *UBE3A*. When his lab-designed virus infects a cell, instead of producing copies of the virus, that cell will begin manufacturing the associated protein that patients with AS lack.

The first-ever clinical trial for a gene therapy was performed in 1990 in the Clinical Center at the National Institutes of Health. Since then, more than 2,300 clinical trials have used some form of it, though more than half tested only the treatment's safety and not its effectiveness. While many early clinical trials failed, a number of recent successes have reinvigorated the field. In 2003, a gene therapy for specific forms of cancer was approved for sale in China, while in 2011 a gene therapy for peripheral artery disease entered the market in Russia and in 2012 a gene therapy for a rare genetic condition called lipoprotein lipase deficiency (LPLD) became available in Europe. More recently, on May 27, 2016, the European Commission approved a gene therapy for children with a genetic condition called adenosine deaminase deficiency. And while the U.S. Food and Drug Administration (FDA) has not yet approved any gene therapies for the American health care market, a treatment for a rare genetic eye disease called Leber's congenital amaurosis (LCA) may soon become the first.

The LCA treatment involves injecting a type of virus called an adeno-associated virus (AAV) directly into the eye in order to deliver the properly functioning gene. First discovered in the 1960's, AAVs have recently been adopted into the emerging field of gene therapy because these viruses do not make people sick or provoke a reaction from their immune systems. They also can be tailored to target only specific types of cells and insert their genetic material into those cells in a more predictable fashion than other viruses. As a result, AAVs have been used in over 117 clinical trials around the world for diseases such as spinal muscular atrophy, hemophilia, and Parkinson's disease. So rather than trying to rev up CAMKII in humans, something scientists currently do not know how to do, Weeber now hopes to treat Angelman Syndrome by using an AAV to place a functioning copy of the *UBE3A* gene directly into the brain.

In a 2011 study, Weeber and his colleagues inserted a copy of *UBE3A* into the hollowed-out virus and then injected it into the brains of AS mice – specifically the hippocampus, a brain

structure known to be involved in learning and memory. They then tested the animals' capacity to retain new information with a task called the Morris water maze. In this test, the mice had to locate a small, submerged platform in a large pool of opaque water surrounded by physical cues meant to help the mice track their own location in the pool. While initially the mice could only find the tiny island by swimming around haphazardly until they stumbled upon it, they eventually learned to use those cues to figure out where they were in relation to the platform and swim directly to it. But three days after the training period ended, the scientists played a nasty trick on the mice: they removed the platform and watched the mice swim around the pool in a desperate attempt to find dry land that was no longer there. Remarkably, the Angelman mice given the AAV treatment swam to where the platform used to be just as often as genetically normal mice, while control Angelman mice given a non-therapeutic AAV that did not contain the *UBE3A* gene appeared to have no idea where the platform used to be. Instead, they swam aimlessly around the pool for the entire test.

The gene therapy also improved the AS mice's performance on another test of learning and memory and partially boosted a neurological process critical for learning that happens less in the brains of AS mice and human patients. Perhaps most importantly, the treated mice ended up with just as much of the UBE3A protein in the hippocampus as genetically normal mice. And because these were fully grown animals that had lacked the *UBE3A* gene since birth, the results seemed to confirm what the Angelman community already suspected: that humans with AS do not have to be treated in utero to see benefits. A dose of Weeber's gene therapy, it seems, might help Angels of all ages.

Weeber and fellow USF neuroscientist Dr. Kevin Nash are now working with Massachusetts-based Agilis Biotherapeutics, a company focused on developing AAV-based gene therapies for rare neurological diseases, to move this work from the lab to the clinic. But before he can test out his approach in humans, several issues need to be worked out. For one thing, a human brain is much larger than a mouse brain, necessitating a larger amount of virus and possibly an entirely different method of introducing the virus into the brain in order to affect a much larger number of neurons. "A mouse brain is just about the size of an almond, and we were just targeting a little section of that almond: the hippocampus," Weeber said. "So how do you get that virus everywhere in the brain without having to give a child 500 injections into the brain?"

Weeber theorizes that the AAV could be injected into a liquid-filled cavity of the brain called the lateral ventricle that is more accessible than an area like the hippocampus. The liquid in this chamber, called cerebrospinal fluid, could help carry the virus to neurons all over the brain. The treatment could also be injected into the spinal cord, a fairly common procedure, or even into a vein if a virus could be developed that can pass through the blood-brain barrier. Weeber also hopes to create and test a modified approach to his gene therapy that turns the affected neurons into "protein factories" that not only make the UBE3A protein for themselves but also ship it out to their neighbors.

Once Weeber and Agilis settle on the specific form and delivery method for the gene therapy, they will still need to perform a number of "pre-clinical" studies before the company can begin testing it in humans. Typically, new therapeutics are first tested in cell and mouse models of a disease in order to verify that they affect disease-relevant processes or improve some symptoms. Next, the treatment is tested for safety in a healthy, larger animal, such as a dog or a pig, whose body is more similar in size and design to a human's. Only after these "pre-clinical" animal studies are done can clinical trials in humans begin.

The first of these, known as Phase I trials, focus only on determining the treatment's safety and side effects in a small group of healthy volunteers. Once safety is confirmed, researchers move on to Phase II and III trials, in which they give the therapy to increasingly large groups of patients in order to determine the optimal dose, evaluate its efficacy, and further gauge its safety and side effects. But because of the unique safety issues involved in gene therapy, Agilis' first human tests will be a combination Phase I/Phase II trial with Angelman patients rather than healthy individuals. In the United States, once a treatment is confirmed in a Phase III trial to be safe and effective, a pharmaceutical company submits its data to the U.S. Food and Drug Administration (FDA), which then makes a decision about whether the treatment's benefits outweigh its risks to the point that it should become available for all patients with the target condition.

Because of the large number of steps in this process, it can be difficult to guess how long it will take for a treatment to make it to a patient population. "That's the million-dollar question," said Dr. Jodi Cook, Agilis' chief operating officer. "It's a hard guess because it depends on how each one of the studies comes out." Still, Cook believes that Agilis will be able to begin Phase I

trials for Weeber's gene therapy within the next two years. At that point, the future becomes more difficult to predict, with Cook estimating that Phase II and III trials will take five to ten years to complete.

Regardless, the involvement of a pharmaceutical company like Agilis will likely hasten the process dramatically, compared to Weeber and organizations like FAST and the ASF going through it on their own. "They're a business," Weeber said. "They can't drag their feet like researchers can. They have a board. They have investors. They need to move quickly on this." Indeed, in 2015 Agilis received the FDA's Orphan Drug Designation for its AS gene therapy, which – among many other benefits, including copious financial incentives – makes the treatment eligible for an expedited FDA review process once clinical trials have concluded. The hopes of the Angelman community are also buoyed by the success Agilis has seen with similar treatments it has created for other diseases. A before-and-after video of a patient with a genetic condition called AADC, which causes marked defects in motor control and strength, elicited audible gasps and roaring applause from the audience at FAST's 2016 Science Summit. The video began with the patient, a two-year-old Asian boy, laying practically motionless on a rubber mat with a feeding tube up his nose. It then cut to footage of the same boy one year after receiving a single dose of gene therapy, which showed him sitting up and playing peekaboo, his feeding tube nowhere in sight.

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Ed Weeber is far from the only researcher hoping to treat Angelman Syndrome by targeting the *UBE3A* gene, nor is Agilis the only pharmaceutical company getting in on the action. Most of these other efforts are taking a markedly different approach inspired by the work of Dr. Ben Philpot, a researcher at the University of North Carolina-Chapel Hill. In 2012, Philpot published an examination of how thousands of different chemicals affect the inactive paternal copy of *UBE3A*. He ultimately discovered that a class of compounds sometimes used for cancer chemotherapy, called topoisomerase inhibitors, turned the gene back on in neurons cultured in the lab, as well as in the spinal cord neurons of living AS mice. But while those molecules could potentially serve as a treatment themselves, concerns about toxicity (chemotherapy drugs destroy both healthy and cancerous cells) have led others to investigate alternative approaches with the same end result.

One of these scientists is Dr. Linyan Meng, an assistant professor of molecular and human genetics at Baylor College of Medicine and a former PhD student in the lab of Dr. Arthur Beaudet, the Baylor professor who first identified Angelman Syndrome's genetic basis. In an April 2013 presentation of her graduate research, Meng explained how, in a mouse model of AS, she had introduced a special DNA sequence into one strand of the paternal *UBE3A* gene. The data suggested that her manipulation halted the process that turns off that copy, just like Philpot's topoisomerase inhibitors.

Afterwards, a Baylor professor who had listened to Meng's presentation approached Beaudet to propose he contact a company the professor had previously worked for. That corporation, now called Ionis Pharmaceuticals, specializes in developing treatments for genetic diseases using molecules called antisense oligonucleotides – "ASOs" or "oligos" for short – that are put together in a lab from the same subunits that make up DNA. Like Meng's approach, these compounds have the potential to restore the function of the paternal *UBE3A* gene, but in a manner much more feasible in humans than the kind of complex genetic alteration Meng performed in her mice. ASOs would also likely be safer than toxic chemicals like Philpot's topoisomerase inhibitors because the specific sequence of subunits used to construct them can be tailored to target a specific gene, thereby reducing the treatment's side effects.

At that time, Ionis had developed ASO treatments for numerous genetic diseases but had not yet applied the technology to Angelman Syndrome. Several months and numerous phone conversations later, the company sent Beaudet's lab a variety of ASOs to test on neurons from genetically altered *UBE3A* "reporter" mice whose cells produce a glowing molecule when the paternal *UBE3A* gene is active. "We were very excited," Beaudet said. "They provided us with hundreds of free oligos – they had that part of the technology up and running. And we had the mice – mice that were especially marked in a way that was good for screening these oligos."

Meng and Beaudet quickly discovered that some of the ASOs could turn on the paternal copy of *UBE3A* in neurons that had been extracted from those animals' brains. Next, Meng and Beaudet, along with scientists at Ionis, began testing the compounds in two different AS mouse models, ultimately showing that the molecules significantly boosted levels of the UBE3A protein for up to 16 weeks in multiple areas of their brains, though not quite to the levels seen in genetically normal mice. In addition, AS mouse models (though not always human Angels) are

prone to be overweight, and the treated mice showed reductions in body weight. They also performed better on a test of learning and memory. However, other behavioral tests and tests of the animals' motor coordination failed to find significant improvements after the treatment. But this doesn't concern Beaudet, who believes the treatment could proceed to Phase I clinical trials even if it fails to significantly improve symptoms in AS mice, which can be difficult to measure. Since those symptoms occur because the paternal copy of *UBE3A* is turned off, if the ASOs activate the gene, "it's pretty predictable that that's going to be beneficial," Beaudet said.

But before clinical trials can begin, Beaudet's team must develop a "humanized" mouse to test the ASOs on, since the compounds tested so far have been designed to target the mouse version of the *UBE3A* gene, which is similar to the human gene but not identical. This is proving to be a difficult feat. "There's been enormous strides in being able to manipulate mouse genomes, but it's not completely straightforward to do certain kinds of more complex manipulations" like this one, Beaudet said. After a year passed with little success, Beaudet submitted a grant proposal to FAST in the fall of 2016 so that he could devote more resources to the project.

Even without seeing data in a humanized mouse, several factors bode well for the usefulness of ASOs in treating Angelman Syndrome. For one thing, similar treatments for other diseases have already been given FDA approval. In December 2016, the FDA approved an ASO therapy that Ionis had developed for spinal muscular atrophy. In addition, there may be fewer safety issues to work out in an ASO-based therapy than a gene therapy, since a paternal copy of *UBE3A* that has been turned back on is more likely to behave in a natural way in the brain than a new, lab-designed gene placed into the brain via gene therapy. "In certain cells *UBE3A* is turned on to a high level and in other cells it's turn on at a low level," Beaudet said. "We think a lot of that would be preserved, but we don't know a thousand percent that would be the case."

Bennett's lab at Ionis and Beaudet's at Baylor are now trying to develop a finalized ASO molecule that has the greatest effect on *UBE3A* with the fewest side effects. "Our hope is in the next couple of years we'll have a drug to go forward into clinical trials," said Dr. C. Frank Bennett, Ionis' senior vice president of research. The trials themselves might take another five to seven years. But as with Agilis' gene therapy, it's impossible to say precisely when an ASO

treatment might become available for Angels. "That's what we're working towards," Bennet said, "but science doesn't always cooperate."

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It's Saturday night, the final night of the 2016 FAST Summit, and a thousand people have gathered for the headline event: the annual FAST Gala. Dressed in their finest evening wear, the attendees have taken their seats in the hosting hotel's elaborately decorated grand ballroom. Dozens of tables adorned with blue tablecloths are spread around the area, and a sizeable dance floor abuts the stage where Allyson Berent now stands behind a podium and a microphone, telling nearly the same story about her daughter Quincy that many in the room could probably tell about their own children.

The neurologist first told her that Quincy would never walk. "I could handle that," Berent said. "She would never talk – that was hard for me. She would never go to college – that was more difficult. She would never get married. She would never live an independent life. She would never have children of her own. All of the dreams of this beautiful little baby were taken away from us with one phone call." She described how she fell to the floor, crying, and her other daughter, two and a half years old at the time, asked her, "Mommy do you need Band-Aids for your eyes?"

"It was at that moment that I realized that this baby – this seven-month-old baby – will say that one day, and I will not stop until she can," Berent continues. "I cannot wait to walk into the neurologist's office…to say to him, 'Don't you ever say never.'"

But as with any other disease, finding an effective therapy for AS is a slow and expensive process. FAST's goal, Berent announces, is to help move such a treatment into clinical trials by the end of 2018, but to do that they need to raise $2.5 million by the end of 2016. And so she begins the annual paddle raise, asking those in the audience to pledge donations of progressively smaller amounts. Berent starts off asking for $350,000, and a single man stands and holds up his number to thunderous applause. The next request is for $250,000, and again someone heeds the call. By the time Berent has gotten down to $100, it seems as if nearly everyone in the room has raised their number at some point.

In the span of just twenty minutes, FAST has managed to raise over $1.1 million. They may not have reached their goal in one night, but even if they had, their work would not nearly have been done. Curing a genetic disease – even one with a relatively simple, single gene involved like AS – takes decades of lab work followed by years of clinical trials and government review. And at any point along the way, a promising approach could be derailed by a lack of funds, scientific or industrial disinterest, unwelcome side effects, or weak evidence for efficacy in animals or human patients. But with gene manipulation improving at an unprecedented rate and several promising approaches to targeting the *UBE3A* gene being developed, a meaningful treatment for Angelman Syndrome is looking increasingly to be just a matter of time.

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**Interviews**

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Arthur Beaudet, in-person interview, 12/2/2016
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Athena Jimenez, in-person interview, 12/2/2016

Benjamin Philpot, phone interview, 12/30/2016

Charlie Williams, phone interview, 10/28/2016

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Donna Young, Facebook interview, 3/12/2017

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Michael Cecere, phone interview, 12/21/2016

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Scott Dindot, phone interview, 11/22/2016

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Robin Clark, in-person interview, 12/1/2016