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A family's history with cystic fibrosis

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Cystic fibrosis took five of her siblings at a young age. Now, Wanda O'Neal, PhD, is part of a team of UNC researchers searching for reasons why. Their latest work has provided new insights that will help unravel why a sixth sibling with CF is living a productive life as he turns 50.

Wanda (Lemna) O'Neal was four years old when her sister Nancy died.

"I remember my father got a call from the hospital," O'Neal said. "It was pneumonia, they told me. They said she choked."

This was 1966. Nancy was only eight years old, though she'd been sick most of her life. Doctors suspected that Nancy had cystic fibrosis, but they didn't know what caused the disease. They didn't know how to treat it. They didn't know what to tell parents.

The family doctor in Enderlin, North Dakota told Mrs. Lemna, "I think you should get all your kids tested for cystic fibrosis. There's a simple sweat test."

Her parents complied.

"I remember that day," said O'Neal, now an associate professor of medicine in the UNC School of Medicine. "It was sad and surreal. We all took our turns going in for the test. My mother kept finding out that another one of her kids had CF. I know that she knew before they told her, because we all knew which kids were thin and which ones coughed a lot. But she still cried."

Of the eight Lemna children born at the time, six were diagnosed with the disease. As a result, the Lemna home was a place full of young kids who coughed through the nights. They struggled breathing. They suffered through lung infections and assorted other health problems. Only Wanda and younger brothers Bill and Jeff, who was born later, didn't have it. Not having CF meant that the surviving children carried the memories of those who were sick.

"My childhood was demarcated by someone dying," O'Neal said. "For 16 years, my mother took care of sick kids, and despite all of her efforts, we watched as each of them died from the same disease in basically the same way."

Except one: Mike.

"He was never really sick, but he definitely had CF," O'Neal said. "It was weird. When my brother Danny died, he was a year younger than Mike but always much sicker. My mom was constantly taking care of Danny and the others, and there was Mike, just hanging out."

Today, Mike is approaching 50, and he's rarely been hospitalized, and not usually for pneumonia. Why he survived is still a mystery. "Something is protecting my brother; something is saving his lungs," said O'Neal. "We don't know what, but what if it's something simple? What if it's the same thing that protects other CF patients who have milder disease? What if we could find out what it is and exploit that knowledge to help protect the lungs of other patients with severe disease?"

These questions are at the center of O'Neal's latest research. She's part of a UNC research team renowned for its basic science and clinical research approaches to developing better treatments, while searching for a cure to CF.

This year, O'Neal and her colleagues at the Marsico Lung Institute / UNC CF Research Center homed in on a potential culprit for the development of severe disease – a group of genes that could become therapeutic targets.

O'Neal said that this discovery doesn't mean they're about to find a magic wand to make CF patients avoid pneumonia. "Science doesn't seem to work that way," she said. "People need to understand the generally slow nature of discovery. But we've found another piece of the puzzle. And that means a lot. It means we know more than we did and we know where to keep looking to understand the disease better."

For O'Neal, being part of a respected team dedicated to improving CF care is a perfect fit and a privilege. But for much of her life – even well into graduate school – she thought the disease that took her five siblings was part of her past, not her future.

Life on the Farm

When she was young, O'Neal never thought she'd become a scientist.

She never thought of becoming a doctor or a researcher. Instead, she found solace in life on the family farm.

"It was rare for a girl to be so interested in farming back then and probably even rarer for a father to let his daughter farm," O'Neal said. "But I was a tomboy. I loved farming, and my dad was really supportive."

She milked cows and took turns on the tractor, helping her dad keep the 600-acre farm viable in a state full of them. When she turned 16, O'Neal's dad got rid of the cows and became a grain farmer. Wheat, corn, sunflowers. O'Neal spent hours on the tractor plowing row after row after row. Sixteen-hour workdays were commonplace, and her work ethic translated into the classroom, where she excelled in all subjects.

Straight A's throughout high school made college a logical choice, and O'Neal became the first person in her family to attend. She chose North Dakota State, an hour away in Fargo. Her favorite subject was biology – plant pathology to be specific. She loved learning about plant diseases, and she figured such a thing would fit in with her family's needs back on the farm. She added crop science as a second. It was during those classes when O'Neal was first introduced to the field of genetics.

"I just loved the subject," she recalled, "even though I was focused on the methods for breeding crops back then. But fundamentally, plant genetics is not much different than human genetics."

She often thought of her siblings and the nebulous idea that she shared similar genetics to them, even though she and two of her brothers were somehow spared getting cystic fibrosis. The last of her siblings with severe CF, Danny, died when O'Neal was a junior at NDSU.

In 1984, with a bachelor's of science under her belt, O'Neal pursued a

master's in crop science. Her parents were puzzled; why the need for a master's in something called "crop science?"

"My family didn't understand me, and I was struggling to figure out what I wanted to do. To them, I was, 'all brains and no common sense.'"

After two years, and still enamored with academia, she was accepted into NDSU's crop science PhD program. She loved the pathology, the science behind how crops got disease or stayed healthy. Learning became a greater love than farming. But still, prior to starting the doctorate program, she confided to a friend that she didn't know what she'd do with another degree in crop science.

That friend then asked a simple question: "Well, Wanda, forget about crop science for a minute; what do you really want to do with your life?"

Without thinking, O'Neal blurted out, "What I really want to do is work on CF."

Back then – because of where she had come from, her family, her farm – the notion of becoming a biomedical researcher had never crossed her mind, not even as a dream possibility.

"I had no one to talk to about it," O'Neal said. "No guidance. No college professors suggesting anything like it. And I had no idea how to even go about it. I was clueless. But it made total sense – the genetics of it, my family, my love of biology. Once I thought of it, there was no turning back."

Life at the Bench

Soon after, O'Neal went to the library to scan through the thick books that listed doctorate programs to find a program with faculty that worked

on cystic fibrosis. She was accepted into the University of Pennsylvania, the University of Utah, and the Baylor College of Medicine. She chose Baylor, and joined the lab of Arthur Beaudet, MD, a professor of molecular and human genetics.

While applying and interviewing, O'Neal didn't mention her siblings. She didn't mention how Mike was still alive and doing well, while her other siblings had died long before they were old enough to vote.

"But when I joined Dr. Beaudet's lab, I did tell him, and I asked if I could work on CF," O'Neal said. "At first, he was a little hesitant, I think because he had had some experience with people who wanted to do research for personal reasons.

"I think, sometimes, people who take their work really personally think they have to cure something or solve the entire problem quickly," O'Neal said. But science for the most part is a series of small steps. "People can become so emotionally involved that they can't make progress. Or the emotion becomes a distraction, and that makes it harder for them to function."

But O'Neal could focus. At Baylor, she continued her success in the classroom, and the girl from the North Dakota farm found a new home. And Beaudet noticed O'Neal's workman-like attitude. In the lab, she took well to the drudgery of repetitive experiments. They were like those long days plowing the farm; she didn't mind.

"I think I was able to do tasks that maybe other people – well, they didn't really want to do them. I didn't feel like I was out of place doing anything. Dr. Beaudet accepted me."

Not long after she joined the lab in the fall of 1988, scientists discovered the gene responsible for CF. They called it CFTR. Scientists cloned the

gene. They realized that people could carry one faulty copy of the gene – as O'Neal's parents did – and if each child acquired one faulty copy from each parent, then the result was cystic fibrosis.

"My parents were unlucky," O'Neal said. "The odds were that one-quarter of their kids would have no good copies of the CFTR gene, half would have just one copy of the CFTR gene and be healthy, and one-quarter would have both copies. So, odds were that only two or three kids would get both copies and get CF. Instead, six did."

The Lemna kids, Christmas 1964: (from left) Wanda, Ron, Nancy, and Joanne. All but Wanda died from cystic fibrosis when they were children.

But still there was Mike. Two copies of the CFTR gene, but he didn't die. He never even got pneumonia. And as researchers would learn soon after the discovery of the CF gene, there were other people with CF who inherited both copies of the faulty CFTR gene without experiencing severe disease.

Researchers began parsing the intricacies of CF biology as they searched for a cure. Eventually, they found that the CFTR gene could be mutated in different ways.

Beaudet, noticing O'Neal's work ethic, put her in charge of genotyping all the DNA his lab had acquired from CF patients in search of those that carried the delta-F508 mutation, which turned out to be the most common CFTR mutation – the underlying reason O'Neal's brothers and sisters had died.

In those days, with the gene in hand, researchers thought a cure was within reach. Then, when scientists introduced the concept of gene therapy as the newest big idea in biomedical science, cystic fibrosis researchers thought they had a clear route to a cure.

The Complicated Truth

The idea of gene therapy is simple. If there's a disease in which only one gene is faulty – like CF, sickle cell disease, Huntington's disease – then we ought to be able to replace that gene with a working copy. But single-gene diseases turned out to be more complex to treat with gene therapy than people originally hoped, especially for CF.

To transmit working copies of the CFTR gene to the lungs of people with CF, scientists needed a vehicle – something to carry the genes to the place they needed to go. The best candidate in the 1990s and 2000s was a rejiggered virus. The idea was to scrape out the viral genes that made

the virus harmful and add the CFTR genes. Then scientists would introduce the CFTR viral vector into the lungs and the new genes would properly express the CFTR proteins, which would play their part in maintaining proper pulmonary health.

It didn't work, and there were probably several reasons.

"With gene therapy, researchers were trying to target cells in the airway – epithelial cells, which are designed to prevent things from getting into them," O'Neal said. "The whole purpose of these cells is to protect the body, to clear junk from the lungs, to provide a barrier so nothing gets through them."

Inside our lungs is a layer of mucus that traps all kinds of particles we breathe in every minute of every day. This mucus layer slides on a watery layer, beneath which lie the epithelial cells that move the mucus, and all those particles, out of the lungs.

We rarely think about this natural process, until we get sick and wrongfully blame mucus for our woes. But CF patients think about it all the time, because the faulty CFTR gene makes mucus thicker and more difficult to remove. In CF patients, the mucus still traps particles. But some of the particles, such as Pseudomonas, fester and cause infection because the CF lung can't move out the mucus in a normal way.

Getting a viral particle to effectively infiltrate this protective system has thus far proved too difficult.

"The mucosal layer is just very protective," O'Neal said. "And if something would get through, then the cells would sense it and do everything they could to get rid of it." It didn't matter if that "something" was a harmless virus carrying potentially life-saving cargo.

Gene therapy for CF never came to fruition, though there is a new call for research proposals to use gene therapy to treat CF because it theoretically makes so much sense. "But so far no one's been able to figure out an efficient way to get the DNA into the cells," O'Neal said.

As gene therapy wasn't panning out, CF researchers continued to develop better ways to treat patients, including physical therapies, antibiotics, and improved methods for keeping the airways clear.

Also, researchers began learning more about different CFTR mutations. They've now identified about 1,800 different mutations to the same gene, including the most common one: the Delta F508 mutation.

It's easy to understand why people with different mutations experienced different levels of disease severity. But why did every member of O'Neal's family suffer from severe CF, except Mike?

Researchers suspected that the clues were in the DNA of the cells at the center of the disease.

Carolina Connection

At Baylor, O'Neal genotyped all of the DNA samples Beaudet's team collected from CF patients to identify those with the Delta F508 mutation. She found that about 80 percent of CF patients had the Delta F508 mutation. She also found that this mutation happened much less frequently in people with Jewish ancestry.

As a graduate student, O'Neal co-wrote a paper for the *New England Journal of Medicine*. She was listed as the first author.

"It was funny, because everyone was saying this was a big deal," O'Neal remembered. "But I was pretty clueless. Later on, I realized that being

first author of a paper in a journal of that stature was really a major accomplishment. In a way, it was good to be naïve. At the time, I just wanted to help Dr. Beaudet, because he was helping me."

O'Neal stayed on for a postdoctoral fellowship at Baylor, where her husband was finishing his residency. Toward the end of their time in Texas, her mentor phoned a colleague in Chapel Hill to put in a good word. That colleague was Richard Boucher, MD, whose UNC research team had created the first mouse model of CF. That research was published in *Science* just as O'Neal's mouse models of CF were being born at Baylor. She was beaten to the punch, but still managed to publish three papers on her work, including her dissertation. Impressed, Boucher invited O'Neal to join UNC's burgeoning CF Center as a research associate. As a molecular geneticist, she started to work with a variety of faculty members – biochemists, cell biologists, mucus experts, microbiologists, geneticists. She settled into life at UNC in the late 1990s, and became director of the UNC CF Center Molecular Biology Core in 2002.

A co-author on more than fifty scientific papers, O'Neal has been part of a UNC research operation that has become a leader in defining the biology and genetics of the CF lung, testing potential therapies, searching for cures, and creating new treatments, such as hypertonic saline.

One of her main collaborators became Michael Knowles, MD, professor of pulmonary and critical care medicine, who for a decade has spearheaded an effort to gather thousands of genetic and blood cell samples from CF patients across the country. One of his goals has been to identify genes and cellular proteins that have subtle effects inside cells that produce dramatic differences in disease severity.

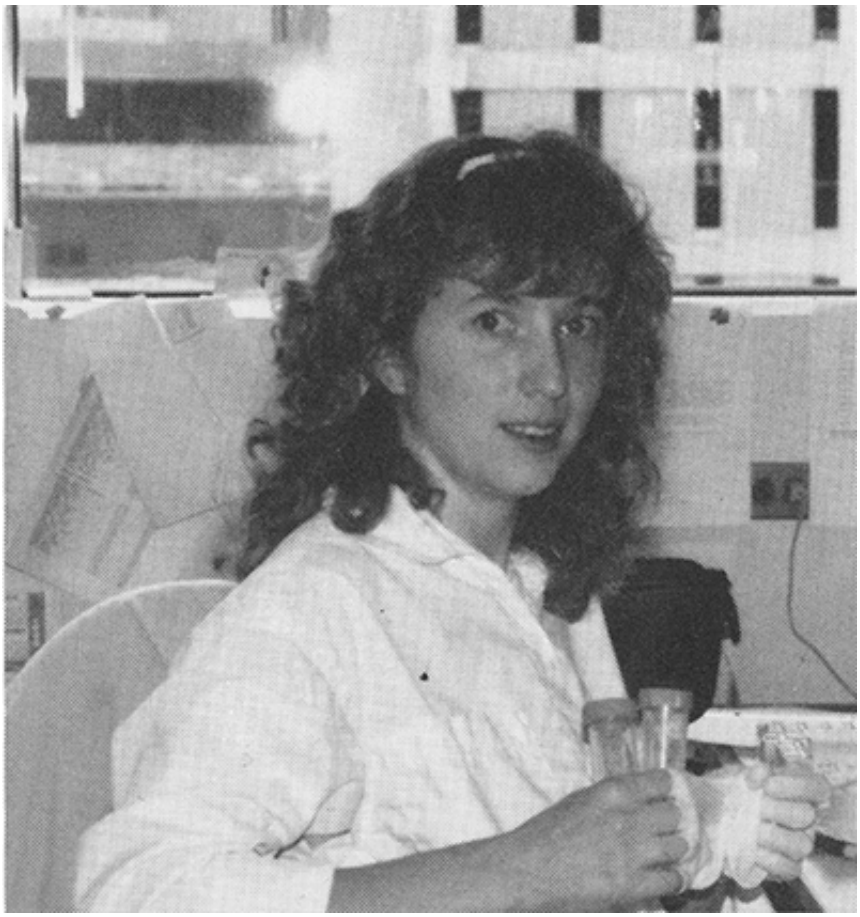
Decades of research on the functions of proteins have allowed scientists

to group genes into pathways based on the common biological roles of those proteins. Scientists deduced that some of these pathways should interact with the main protein created by the CFTR gene.

This is how CF biology works:

In a normal epithelial cell, the CFTR gene creates a crucial protein that transits from the cell nucleus to the cell membrane, where it then works to maintain proper lung function. As the protein transits from nucleus to membrane, there are many genes that interact with it in various ways – creating a genetic pathway – so that the protein can complete the journey and work properly in the end.

In CF patients with the Delta F508 mutation, the CFTR gene does not fold into its correct form and cannot make it to the cell surface. It turns out that the Delta F508 protein doesn't simply make its journey on its own. Nor does it manage to work properly on its own. Other genes are involved. Other pathways.



Wanda O'Neal in the lab at Baylor College of Medicine

Paths toward treatments

For their most recent study of these genes, Knowles, O'Neal, and a group of other researchers used gene expression data from cells collected from 750 patients gathered over the past decade from 40 sites across the United States. Along with NC State biostatistician, Fred Wright, PhD, and others, Knowles and O'Neal were able to analyze more than 4,000 genetic pathways to find the ones that played roles in severe or mild CF disease.

This type of study allowed them to find significant genetic variation in two broad types of pathways: endomembrane pathways and HLA

pathways.

It turned out that endomembrane genes were previously shown to be responsible for transporting the Delta F508 protein from the cell nucleus to the cell membrane and for regulating the way that proteins such as CFTR are folded into the proper functioning form.

The HLA genes, meanwhile, were found to have roles in immune function; they're important for protection against pathogens, such as Pseudomonas – the commonly seen bacteria that cause pneumonia in CF patients.

According to Knowles and O'Neal, disease severity partially depends on how genes in these pathways function. They discovered that when these pathways or groups of genes were highly expressed – when there was a larger amount of proteins produced by these genes – then CF patients had less severe symptoms. When genes in these pathways were expressed in lower amounts, patients experienced a more severe form of the disease and were more likely to be hospitalized.

"Now that we've found these pathways, we need to dig into the biology to see how specific genes within the pathways influence disease severity," O'Neal said. "We hope we can target these genes to limit the severity of disease. If so, this could help all patients in a big way."

The work could also provide important clues for why new CF treatments work well for some patients but not for others.

In 2012, a drug called Kalydeco came on the market. It was considered a miracle drug for CF patients who had specific kinds of mutations responsible for about 4-5 percent of all CF cases. It does not work for the majority of patients – those with the Delta F508 mutation. The drug is also extremely expensive.

Last year, a combination therapy of two drugs showed that it could improve lung function in patients with Delta F508 mutations, but in clinical trials the beneficial effects were modest for most patients. Some patients didn't experience any benefit.

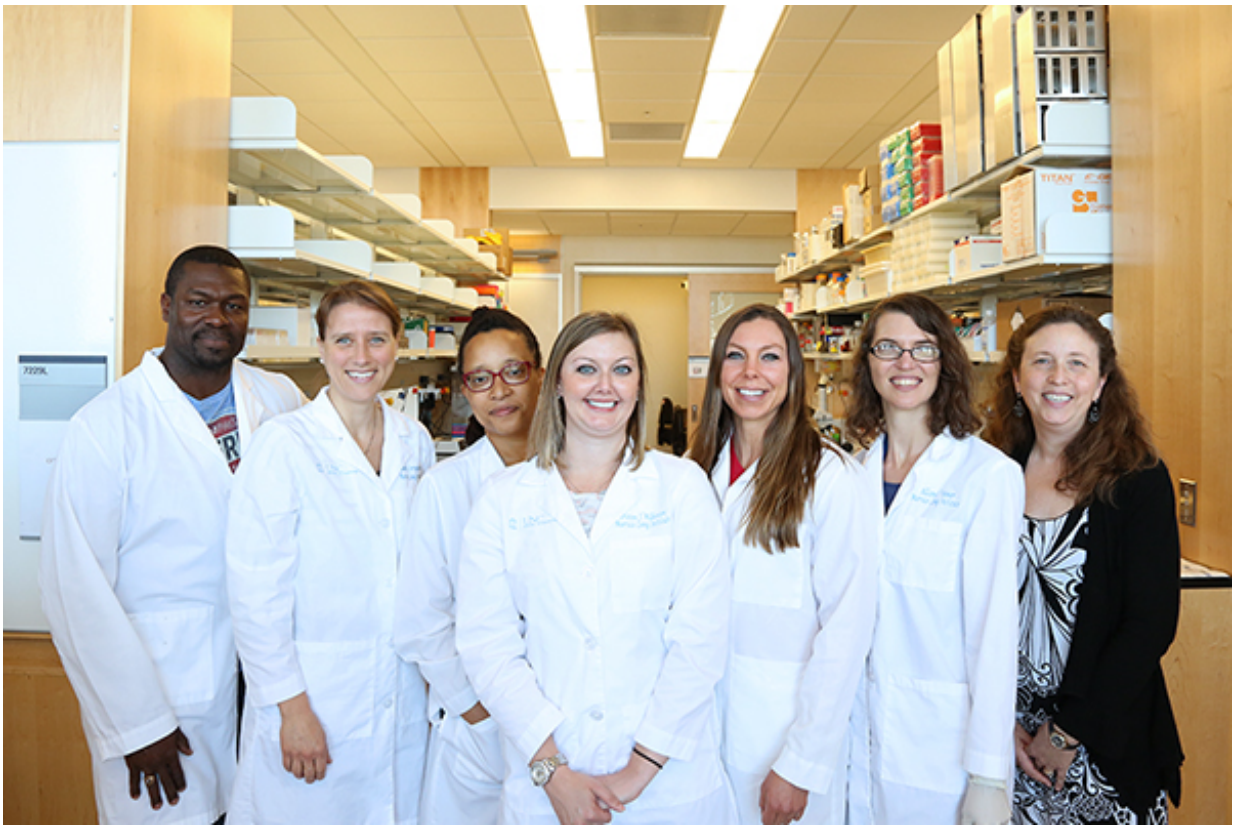
"Why is that?" Knowles said. "Well, it could be that these genetic pathways we've identified play roles not only in severity of disease but whether a person responds to specific therapies, including these new drugs."

If Knowles and O'Neal had DNA samples of the people who didn't respond to the new drugs, then researchers could compare the genetic variation between patients.

"We could perhaps know why some people responded to the drugs while others didn't," O'Neal said. "We could potentially use that information to figure out which patients would respond to the new therapy and which ones wouldn't."

Knowles said, "We really want to know if people who respond well have higher expression of these genetic pathways we've identified. If so, then we're really on the heels of personalized approaches to treating CF patients at the level of their genes to lessen the severity of often debilitating symptoms.

"There's a movement, nationally, to ask drug companies to make these samples available," Knowles added. "It's something we can't entirely control, but it's a potential source of valuable information."



O'Neal Lab (L to R) Rodney Gilmore, Alessandra Livraghi-Butrico, Kristy Terrell, Kristin Wilkinson, Lisa Jones, Allison Volmer and Wanda O'Neal. Credit: Max Englund, UNC Health Care

What the researchers can control is access to DNA and blood from CF patients who have donated thousands of samples to Knowles, O'Neal, and other researchers over the past decade.

The collaborations between researchers, patients, the National Institutes of Health, and the Cystic Fibrosis Foundation have turned cystic fibrosis into a disease that can be managed well into adulthood in many cases, and often patients live decades longer than did children born in the mid-20th century.

Pieces of the Puzzle

When Kalydeco came out, O'Neal called her brother Mike to tell him. He still had never been hospitalized for pneumonia but he had been for intestinal obstruction, which is a common problem for adults with cystic fibrosis. She told him, "Look, if you have a certain kind of genetic mutation, you might want to consider this new drug. We need to test you."

Mike agreed. He didn't have one of the rare mutations that Kalydeco addresses. He had the classic Delta F508 mutation.

"I don't know if we'll ever know why Mike, per se, has been protected against severe disease," O'Neal said. "But now we have identified a few group of genes that the research community can explore to figure out what might trigger severe disease – or protect against it. And it could be possible to manipulate these genes so that all CF patients could benefit."

When O'Neal's siblings were diagnosed in the 1960s, most patients didn't live past their elementary school years. Now, half of all CF patients in the United States are older than 18, but much more work needs to be done.

When O'Neal began working on CF in the 1980s and especially when the CFTR gene was discovered in 1989 and scientists thought gene therapy could be the answer, she and others hoped that they could be curing patients by the end of the 20th century. It turned out that a simple single-gene disease was more complicated.

Finding better treatments and cures took large groups of scientists, dedicated patients and parents, drug companies, and substantial investment from funding agencies – especially the NIH and the Cystic Fibrosis Foundation – and individuals such as Thomas Marsico.

Kalydeco, for instance, happened after decades of basic research that cost millions of dollars; the clinical trial got the press that it deserved, but it would've been impossible to achieve clinical success without basic lab research.

In basic science, O'Neal found her calling.

"I'm very happy to be a piece of the puzzle. As director of the molecular core here at the CF Center, I'm in a position that allows me to help many people in the research community," O'Neal said. "Really, with almost anything related to molecular genetics, our core can help. This is a great fit for me."

If scientists need to know the level of expression of the CFTR gene in specific cells, O'Neal can find out. If they need RNA or DNA analyzed, they go to the molecular core. If they need a mouse model, O'Neal will make it happen. If they need to test a drug on cells or mice, they talk to O'Neal. If they need to know how a drug interacts with specific proteins, they work with O'Neal.

And as someone who lost five siblings to the same disease, O'Neal knows what's at stake for patients and their families.

"It's important for families to know that there are people working on this disease and that we care about it," she said. "It's important to be able to say to your child that there might be a cure – not today or tomorrow – but that good people are working on it and that there is hope. I honestly don't think this is false hope. I think it's real, because we've come far since I was a kid. I don't ever want to lose sight of that."

Provided by University of North Carolina at Chapel Hill School of Medicine