CUSTOMER SPOTLIGHT

Deciphering Feline Mutations Through Whole Exome Sequencing with Twist

Feline Genomics Spotlight with Leslie Lyons, Ph.D.

Researchers studying organisms that are not traditional model systems face an uphill battle. These organisms often lack specifically designed research resources, tools, and reagents that improve the efficiency and quality of experiments in species like humans and rodents. Still, studies into diverse organisms can have far-reaching impacts, thanks to the intrepid researchers willing to blaze new trails despite less infrastructure, commercial products, and funding.

In this interview, Twist sat down with Leslie Lyons, Ph.D., a professor and researcher studying one such model organism: the cat! Leslie leads a research team at the University of Missouri-Columbia and heads up the Feline Genetics and Comparative Medicine Lab. Leslie and her lab applied a custom exome capture panel and target enrichment reagents from Twist to further profile the cat genome and identify mutations with both animal and human health implications.

TWIST BIOSCIENCE

How did you get onto your research path, and when did you realize your passion for studying cats?

My research career started as an undergraduate at the University of Pittsburgh. Though I was getting a biochem degree, I was pre-vet and planned on attending the University of Pennsylvania after collecting my bachelor's. But, to attend U. Penn's School of Veterinary Medicine, you had to take genetics as a prerequisite. At the time, there was only one undergraduate class in genetics at Pitt. So, I took it and was immediately hooked on genetics. I then decided to take graduate-level genetics courses as an undergraduate just to learn more. Through those courses, I met my research mentor, Robert E. Ferrell, and started working in the lab. I realized I liked genetics far more than I wanted to be a veterinarian, so I ended up staying in Robert's lab to do my master's and my Ph.D. There, I studied human genetics, learning how to build genetic maps and find mutations for diseases, including colon cancer and different eye defects. As I worked on my graduate degrees, I decided that I wanted to do more comparative medical genetics and expand beyond humans. So, when I was looking for a post-doc position, I interviewed with a cattle lab, a fish lab, and Steve O'Brien's lab, who studied cats as a model. I ended up in Steve's lab because I still wanted to keep a tie to human genetics while also learning about comparative, evolutionary, and conservation genetics. After deciding that my gene-hunter skills were a good fit, Steve approached me, saying, "I have a cat project I'd like you to lead."

And that was it. I never chose cats. They chose me through Steve. My job was to help build a genetic map for cats. To do this, my first project was to work with cat breeders to create a family through interspecies back-crossing and assisted reproduction. It turns out that my second love was theriogenology!

Why is it important to study feline genetics?

Well, there are a few different reasons. First, the cat offers useful models for inherited human diseases. To get the most medical information, you need the right model for the right disease. We've come to learn that though you can knock genes in and out of a mouse to create a disease model, you often don't end up with the same phenotype. Instead of creating models, you can instead look for animals with naturally inherited diseases similar to our own. Oftentimes, this provides a closer and more relevant comparison. In addition to some close genetics, cats also have established pedigrees, which can support the model development process.

A second reason is to "fix the cat." Cats are a popular companion animal, so there's a vested interest in keeping them healthy. Though we can't fix them outright, we can develop genetic tests that help us identify disease-associated alleles and better control cat breeding to reduce disease incidence.

Third, studying cats can help us better understand population dynamics and domestication. As part of this, we can compare genotype-phenotype relationships in humans and cats to learn more about how mutations and regulators can affect the biology of organisms differently. For example, a shared gene may cause curly coats in cats but cause ectodermal dysplasia in humans.

Assorted cats with different traits. (iStock)

Tell us about your lab's work.

We've kept along the path of investigating inherited diseases. I'm happy to say that my lab has probably identified the most genetic mutations for feline inherited diseases and traits. For example, we found the polycystic kidney disease mutation, which is actually one of the most common genetic diseases in humans. It is even more common than Duchenne muscular dystrophy, cystic fibrosis, and sickle cell combined. That was a great success because the cat was a really good model: same gene, same mode of inheritance, and same variability in the presentation. We've also taken it to the treatment level, where we've been able to show that if you put cats on a ketogenic diet, the cysts will not only stop progressing, but they'll actually regress a little bit. A nutritional component could very well help to control polycystic kidney disease in humans as well.

More recently, our lab expanded our approach to working on precision medicine. There are about 200 disease-relevant DNA mutations known in cats. We can run a panel to know if a cat has these mutations and share that information with pet owners and vets. That includes genetic details like blood type for transfusions and factor XI mutations that cause prolonged clotting times. Both are useful to know before surgery. We can also investigate drug receptor genes like ABC-B1 (also known as MDR-I). Variants of its gene product, P-glycoprotein, can lead to sensitivity and toxicity to drugs like Ivermectin. Knowing the ABC-B1 makeup can help inform safe drug administration.

Basically, my job is to develop genomic resources for the cat and also apply them. As we build resources, we make them accessible to the research and veterinarian communities. As part of this, we're developing and applying whole genome and whole exome sequencing as a tool for precision medicine in veterinary care. That's what we're now promoting at the University of Missouri. We're inviting vets anywhere in the world to send us the DNA of their problem cases. From there, we sequence it and hopefully respond with information about a targeted gene or variant that may inform treatment. The custom exome capture panel we created with Twist really greatly helps us facilitate that.

Can you share a bit about your findings exploring cat genomes using the custom exome panel you built with Twist?

We easily have about a half dozen new DNA mutations that we will ultimately publish, including a new myotonia congenita and a new type of polycystic kidney disease. We're now writing up the first paper based on these results.

"Prince Harryhausen" (Harry). (Leslie Lyons, Ph.D)

We were also able to pick up some slight rearrangements with Twist's exome capture panel that we missed when using an older panel made by another group. This time, Twist helped us develop a really good study design, and we had a really good genome to work off of. Importantly, we captured everything that we found previously, which helped us know the exome panel we built with Twist was doing a good job.

I'm also excited to say that I think we finally have an answer to a question from one of my first projects 30 years ago, and that's definitely because of the Twist exome panel. But I won't say more until that paper is published!

Why should researchers consider exome sequencing?

The lower cost of the exome capture is a nice intermediate for investigating inherited diseases and doing a quick pass at genome sequencing. People often ask, "Why not just do whole genome sequencing at low pass and impute?" My view is that there's still a lot of guesswork to that. It might work on something clearly Mendelian in a specific breed of cat, but that's not where we're headed. We really want to solve more complicated cases. On that front, having the high depth of coverage that you can get with exome capture makes a huge difference.

Along those lines, I think the whole exome capture panel is the best way to look at cancers because you can get much higher coverage when looking for somatic mutations in tumors and circulating blood cells. There have only been a few cancer studies in cats, but now this opens the door to more.

"It was great working with Leslie to build a Feline exome. Exome panels are complex and the Twist team worked closely with her to incorporate a broad spectrum of important targets, including adapting a set of human cancer targets. I am thrilled that she has used the panel to make novel discoveries in feline disease."

– Owen Hardy, Twist Bioscience

What are the next steps and future directions for your research program?

The next great step would be to start tackling more complex traits that do not have Mendelian-type inheritance patterns. Many of the same complex traits that we don't fully understand in human medicine also affect our cats, like obesity, allergies, and asthma. Plus, cats also share environments and exposures with us.

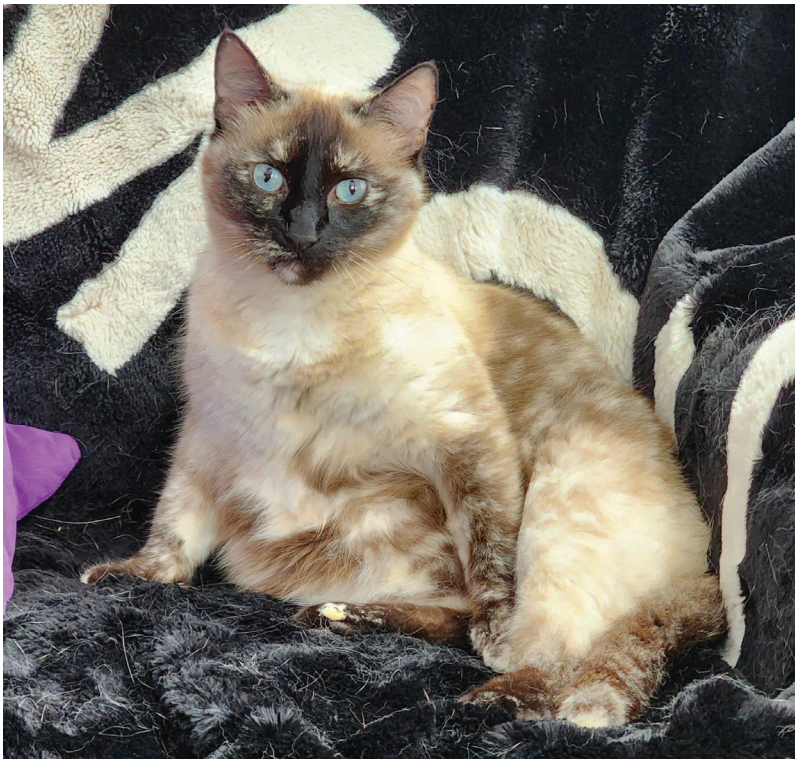
We're also looking at disease susceptibility and resistance to established pathogens like feline infectious peritonitis (FIP, a coronavirus) and emerging ones like H5N1 bird flu. Essentially, the goal is to look for genetic signatures that correlate with transmission and severity.

In addition, I can see another version of our cat exome panel coming down the road. Though we have a really great genome, our annotation is not the best. But we just sent off a lot of data for IsoSeq with the goal of identifying transcripts for gene annotation. With better annotation, we may upgrade the cat exome panel design in the near future.

What advice would you give other researchers considering genomics studies like yours?

Good data in is going to produce good data out, so you have to take the time to do the epidemiology behind whatever you're working on. Is there a heritable component to what you're trying to study in the first place? Are you mixing apples with oranges? There are quite clear-cut diseases that are caused by more than one gene or have variable outcomes. So, take the time to help clarify disease heterogeneity. I worry that a lot of studies just tend to grab things retrospectively instead of trying to prospectively.

My second word of advice is to think deeply about the accuracy and error rates of your genetic tests and the technologies you use. We tend not to consider things like sensitivity and specificity as much in genetic analysis despite their common use in other bioassays. But we should!



"Brat Cat." (Leslie Lyons, Ph.D)

What was your experience building a custom panel with Twist?

I found Twist very helpful and easy to work with. I think it was maybe one of the easiest projects I've done. Twist's really excellent customer service absolutely made a difference. The interaction back-and-forth of "Is this the design you want? What about this? What about that?" helped us refine our approach and make a better custom panel. The Twist team took special care to get us thinking about important design details and to provide their input on things like picking the right sequence spots, base pair overhang lengths, and so on. Part of that is a high degree of transparency. As the investigator, I might not fully understand the intricate details of every technology I use. But, I felt very comfortable that the Twist team was telling me the things that I needed to know instead of making me guess.

What led you to start using Twist's products?

It helped that Twist already had a good reputation. I never buy the "first model of a car," so to speak, and partly that's because the cats don't have a lot of funding. So, there's less room for experimental failure. It was definitely encouraging to see presentations at Plant and Animal Genetics and other meetings that used Twist. I like seeing what products my colleagues are moving to and taking the time to ask them about companies they work with before we get their products.

Having used them yourself, what do you like about Twist enrichment panels?

The NGS target capture products are just very solid technology that works well with Illumina sequencing. You know you're getting nice, good, solid data that everyone knows how to work with.

What are your thoughts on commercial genetic testing for domestic cats?

I think it comes down to how the company presents itself. We know the science can be done well. We also know a fair amount about the relevant DNA variants and which ones are really strong traits with high penetrance and low variability. But I don't like when companies promote things that are just sensational to make a buck. I think that detracts from the work and causes people to lose trust in science.

The only other thing is that it can be challenging for pet owners to understand the results and their certainty. It's important people don't make rash health decisions for their animals based on a large panel assay test or very low-pass sequencing. We try to keep all our sequencing depths at 30x and higher. Sometimes, people try to make their sequencing cheaper or faster by doing it at lower depths. But then your error rate is a lot higher. Personally, I have a hard time justifying that.

"Meow Meow Kitty." (Leslie Lyons, Ph.D)

It's time for a really important question. Do you have cats?

There's my big orange guy, Harry,* short for Prince Harryhausen (after Ray Harryhausen), and his lighter orange brother, Meow Meow Kitty. My female cat is a really soft tortie point, and her name is Brat Cat. But after watching Puss in Boots, she would like to be called Kitty Soft Paws.

*[*Harry, who joined our interview, was recently featured in a Science News story about the gene that causes orange fur in cats]*

Do you have a favorite cat breed?

I get asked that a lot. It depends on who I'm looking at the time. But the Abyssinian is definitely one of my favorites. They're a bit mischievous and high-energy. The Ruddy Abyssinians are just absolutely beautiful. They're the epitome of cats. And actually, we don't know where they're from.

When you're not sequencing and studying cat genomes, what do you like to do in your free time?

Well, I just got back from skiing with friends at Seven Springs, near Pittsburgh. Those same friends also taught me how to scuba dive, and so occasionally, I'll do that. I like to travel. In fact, I was at the Mount Everest base camp in Tibet this year. I also played a lot of sports growing up, like basketball and softball, and I remain a big Pittsburgh sports fan. I've also seen every Major League Baseball stadium in the United States except for Miami.

Oh wow, do you have a favorite park?

Without bias, I think PNC Park in Pittsburgh is one of the best-designed and well-built stadiums.

Thank you to Leslie for discussing your research and feline fascination with us!

Note: this interview was edited for length and clarity.

About

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