

FOR THE AGES

*The Dog Aging Project has One Key Goal:
TO HELP YOUR DOG
and you
LIVE LONGER, HEALTHIER LIVES.*

By Sue M. Copeland

If small dogs live longer than large dogs, why do elephants live longer than mice? Why is age a risk factor for some cancers in dogs and humans? Those are just some of the questions researchers are seeking to answer with the Dog Aging Project (DAP; dogagingproject.org).

DAP is the largest open study on aging in dogs. It's funded by a multi-million-dollar grant from the National Institute of Health (NIH). The goal? To discover new keys to human aging by studying dogs, who share many diseases (such as cancer) and genetic markers with their humans.

What separates DAP from many studies is that *your* dog can be part of it (more about that in a minute). Says the study's founder and Principal Investigator, Daniel Promislow, PhD, Professor, Department of Lab Medicine and Pathology and Department of Biology at University of Washington's School of Medicine, "Our dogs live with us. They drink our tap water. They walk, sleep, and play on our floors, on our furniture, and in our yards. What we learn about them is likely to apply to us."

What would take decades to learn about aging in people can take just a few years in dogs. You can help the Dog Aging Project learn how genes, lifestyle, and environment affect your dog's--and your--aging. Dogs young and old are welcome. For how to join the study, see "Join The Pack!" on the next page.



Says Dr. Ruple, "The entire Dog Aging Project (DAP) should have many benefits for dogs and humans. Our lymphoma study and DAP are about helping both species live longer, healthier lives."

But back to that size question: Why *do* small dogs have longer life spans than large ones? That question planted the seed that sprouted into DAP. "In 2007, a paper was published on the genetics of aging and size in dogs," says Dr. Promislow. "It made the cover of *Science* magazine, which featured a Great Dane and Chihuahua walking side by side. What the paper showed was that while there might be a lot of genes that impact a dog's size, there's a single gene that accounts for about half the size variation you see in breeds. Large breeds have a version of the gene, which they inherited from wolves. Small breeds have a novel version of it that leads to a smaller size," he explains. "Studies have shown that in worms, flies, and mice—and possibly humans—the gene is associated with aging."

As someone who studies the evolutionary genetics of aging in creatures great and small, Dr. Promislow was fascinated. "The problem was, I didn't know anything about dogs or veterinary medicine. At the time I was at the University of Georgia, so I reached out to the veterinary school there. After being passed from person to person, I eventually heard back from a veterinarian named Dr. Kate Creevy. She said, 'I don't know anything about aging, but I'll meet with you anyway.' That was really the beginning." (She's now Chief Veterinary Officer of the Dog Aging Project.)

He continues, "That was in 2008. I started talking about dogs and aging. Dogs are a great model to study because they are 'variable,' meaning there's a lot of breed and size variation. And their lives are sped up compared to humans. What would take decades to learn in people can take just a few years in dogs. The leadership at the Division of Aging Biology at the NIH's National Institute of Aging reached out to me and said, 'Maybe you should put together a research network and do more with this.' One thing led to another. I moved to the University of Washington in Seattle. There, my team and I started building a large base of researchers and veterinarians, which now numbers about 100 people. The idea was to create a nation-wide cohort of thousands of dogs, and to follow them throughout their lives, in their home environ-

ments, to understand how biology and environment shape healthy aging. The goal is to help us understand why some dogs live much longer, healthier lives than others."

A LARGE "PACK"

About 30,000 dogs, of all ages and breeds (including mixed breeds) are currently part of DAP's "Pack." To nominate your dog to be part of the study, you fill out a short survey on DAP's website. (See, "Join The Pack!" below.) More dogs are always wanted.

There are multiple cohorts within DAP. If your dog is chosen to be part of the genotype cohort, known as the "Foundation Cohort," you'll be sent a kit with a cheek swab, which you'll return once you've done the swab. (Instructions are included.) The swab then goes to a sequencing facility, where researchers sequence each Foundation Cohort dog's genome. (The cost is covered by DAP.) Researchers then test whether variations in a dog's genome are associated with variation in the frequency of certain diseases or behaviors, based on the detailed information you'll have provided about your dog.

A second group is known as the "Precision Cohort." Pack members who are chosen for this level of participation get a larger kit sent to them once a year. "The kit includes containers with which the member's veterinarian can collect blood, fecal, and urine samples," explains Dr. Promislow. "A courier picks up the

JOIN THE PACK!

Help the Dog Aging Project learn how genes, lifestyle, and environment affect your dog's—and your—aging. The information will be used to help learn how to increase the "healthspan" (period of life spent free of disease) in dogs and humans.

"We'd love to welcome tens of thousands more dogs to the 'Pack,'" says Dr. Promislow. "We invite all dogs, from puppies through seniors, to join. We also welcome any sex, and purebred or mixed-breed dogs. They can be healthy or have a chronic illness." The only current constraint is that your dog must live in the United States. "We hope to go international with our recruitment in the future," he says. "I call it the power of paying attention. The more dogs we can pay attention to, the more we can learn to help dogs live a long and healthy lifespan."

Here's how to do it: Visit www.dogagingproject.org and click the purple 'Nominate Your Dog' button. You'll be asked to complete a short survey that includes basic information about you and your dog, which will help the DAP team determine how to best include you in their research.

completed kit at the veterinarian's office and ships it back to us. We do standard blood chemistry on the blood sample, a urinalysis, and a fecal analysis. Then we also do more sophisticated analysis of the blood, looking for example at white blood cells and their counts, to collect lots of biological data. All the residual samples are then sent to the DAP's Bio Bank at Cornell University." The data collected is eventually available to researchers around the world, as are the residual samples.

Environmental data is also studied, since environment can be a cancer trigger in dogs and humans. Explains Dr. Promislow, "Healthy aging is the result of both genetics and the environment.

FOUNTAIN OF YOUTH?

Is there a drug that can help our dogs (and us) live longer, healthier lives?

Colorado State University is collaborating on the Dog Aging Project's TRIAD clinical trial to study whether the human immunosuppressant drug rapamycin increases the lifespan of companion dogs. A secondary purpose is to determine whether rapamycin improves various measures of health in aging dogs. The drug has been shown to increase lifespan and delay, or reverse, many age-related disorders in mice.

Says Dr. Promislow, "It's a drug that was discovered in the soil on Easter Island, which is also called Rapa Nui, hence the drug's name. It helps prevent rejection in human organ and bone-marrow transplant patients and is used widely in human medicine. It was discovered that in laboratory organisms such as mice and fruit flies, the drug can increase life span. In fact, it was found that not only did mice live longer when given rapamycin, they lived healthier longer. They have better cognitive function. Their hearts look younger than their age. And the safety profile at a low dose is very good."

He continues, "The goal of the rapamycin study within DAP is to enroll a cohort of dogs in a double-blind, placebo-controlled study. The dogs will be given low doses of rapamycin (or a placebo) once a week and followed for a year. The study will include echocardiograms to look at heart function, and tests to look at cognitive function. The primary outcome will be to look at lifespan: Will treatment with rapamycin for a year lead to a longer, healthier lifespan in dogs?"

Dogs in the study will come from the DAP "Pack," and will have consented to participate, with safety as the priority. (The rapamycin study grant supports about 500 dogs.) "This is being run like a human clinical trial," says Dr. Promislow. "There is a data and safety monitoring board that's independent, at the National Institute of Health, just as there would be for a human clinical trial."

The resulting data could literally be life altering. Says Dr. Promislow, "99 percent of the Dog Aging Project study is telling us what the factors are that can be useful in advancing the healthy lifespan of dogs. The rapamycin study goal is asking, can we intervene in a way to improve a healthy lifespan in dogs with the drug? I think one of the reasons that the National Institute on Aging was excited to support DAP was because of the translational potential for human aging. This is the first clinical trial outside of a laboratory to test the ability of a drug to increase healthy lifespan. If this is successful in dogs, that would certainly encourage interest in studying it in a human population."



DAP's rapamycin study is unique. "This is the first clinical trial outside of a laboratory to test the ability of a drug to increase healthy lifespan," says Dr. Promislow.

It's important for us to study dogs who live in all kinds of settings, from farms to cities."

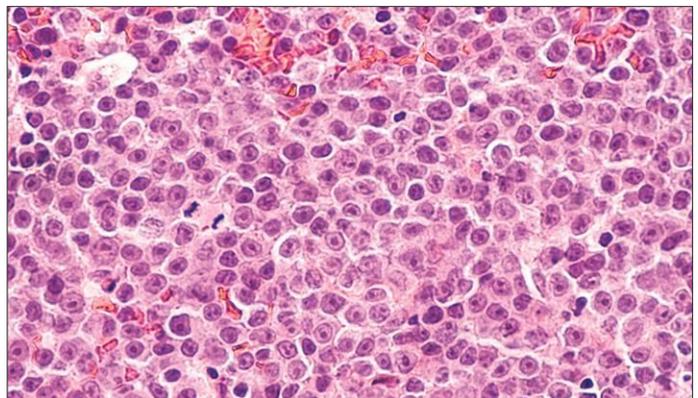
A third group is called the "TRIAD Cohort," which stands for "test of rapamycin in aging dogs." Rapamycin is a drug used in human medicine to help prevent rejection in organ and bone-marrow transplant patients. In laboratory studies using worms, flies, and mice, the drug has been seen to increase life-and health-spans. (For more information, see "Fountain Of Youth?" above.) This cohort represents about one percent of the dogs in the study (about 500 dogs). Most of them will be taken to veterinary teaching hospitals for checkups, where veterinarians will be looking at such factors as heart function, cognition, and lifespan.

Within DAP is an additional study, funded by the AKC Canine Health Foundation (CHF; akcchf.org). Its goal: To search for a blood-based "biomarker" (mutation) for lymphoma, a cancer of the blood's lymphocytes. That could help researchers understand how mutations contribute to a predisposition for the cancer, which in turn will help them understand why certain breeds are at higher risk than others.

THE LYMPHOMA LINK

"What we learn about dogs and lymphoma could help us better understand lymphoma in humans," says Dr. Promislow. "Un-

like humans, who are all genetically unique (except for twins, triplets, etc.), dogs within a breed may not be identical, but from a genetic perspective, they look a lot more like each other than



Lymphoma describes a group of cancers that stem from lymphocytes. "What we learn about dogs and lymphoma could help us better understand lymphoma in humans," says Dr. Promislow.

they do like dogs in other breeds. Because there's a lot of variation in lymphoma risk from breed to breed, we have the power to begin identifying what the genes are, or what the risk factors

FAST FACTS: LYMPHOMA IN DOGS

WHAT IT IS: Lymphoma describes a group of cancers that stem from lymphocytes, which are a type of white blood cell that helps your dog's immune system fight infection. The most common canine lymphoma is diffuse large B-cell lymphoma, an aggressive, fast-growing cancer. A less aggressive form, T-cell, or T-zone lymphoma (TZL) is diagnosed less frequently in dogs.

AGE OF ONSET: It typically is a disease seen in older dogs. Average age of onset (which can vary with breed and size) is between six and nine years old, depending on the type of lymphoma. With B-cell, it's likely to be around six or so years of age. With T-zone, around nine to 11 years of age. "But there can always be exceptions," says Dr. Ruple. "Dogs diagnosed between the ages of two and four tend to have a more aggressive lymphoma than older dogs. That could be because there's more of a genetic predisposition in those dogs, rather than an environmental trigger."

SIGNS OF LYMPHOMA: You'll typically feel enlarged lymph nodes under your dog's jaw, like the ones you get if you have a sore throat. You may also feel them in your dog's arm pits, or behind his knees. Other signs can include lack of appetite, weight loss, lethargy, increased thirst, or just not seeming "right."

TREATMENT: "We typically treat B-cell lymphoma with a multi-agent chemotherapy protocol known as CHOP, on a weekly or bi-weekly basis for several months," says Dr. Ruple. "We can generally treat it into remission in older dogs. That may not mean it's curable, but we may get affected dogs to live long enough to die from other causes. T-zone lymphoma is considered an 'indolent' disease, meaning it's not as aggressive as B-cell; we use a different chemotherapy protocol for it. Dogs tend to respond well to chemotherapeutics—they don't get as sick as humans do. Most tolerate them very well."

BOTTOM LINE: "Lymphoma is not necessarily a death sentence," says Dr. Ruple. "Typically, treatment is more effective the earlier it's diagnosed. But overall, the treatments tend to be effective. A lot of impacted dogs, especially those diagnosed at an older age, can have many more years ahead." "CHF's partnership with the Dog Aging Project to understand risk of lymphoma in dogs embodies the Foundation's mission to improve our dogs' health, and extend their time with us," says Andrea Fiumefreddo, MS, CHF's Vice President of Programs and Operations. "This study will help researchers understand aging and environmental influences at both ends of the leash. We thank all canine participants and their owners for furthering science for a healthier future."



"We think there are genetic predispositions for lymphoma in dogs and humans," says Dr. Ruple. If a genetic marker for it is identified in dogs, it could lead to earlier diagnosis of the cancer. "Typically, treatment is more effective the earlier it's diagnosed," she says.

are, that affect which dogs get lymphoma and which don't. We can use those findings to go back to human research and ask, Does this gene or that environmental factor have an impact on lymphoma risk?" (For how to join the lymphoma study, see, "Fast Facts: Lymphoma In Dogs," above.)

"We think there are genetic predispositions for lymphoma in dogs and humans," says Audrey Ruple, DVM, MS, PhD, DipACVPM, MRCVS, Associate Professor, Quantitative Epidemiology, in the Virginia-Maryland College of Veterinary Medicine at Virginia Tech, who is part of the CHF lymphoma study and a member of the DAP research team. "We also think there's an environmental trigger—we don't see lymphoma in every litter-



Dr. Promislow has high hopes for the DAP. "I think of it as a 'forever project,'" he says. "The dogs we're studying now won't be around in 20 years. I'll be retired in 20 years. But I hope what we've built will still be here, and researchers can take advantage of the infrastructure to learn more about all kinds of things in dogs—and people."

mate, for example, in breeds that have a high instance of it. Lymphoma has been increasing in human populations since the 1960s. We need to learn more about it so we can understand why that's happening—and what we can do to prevent it in dogs and people."

She continues, "In humans, you typically see lymphoma in older white men of Western-European descent; there's also some association with obesity and age. A canine lymphoma study I was part of at Colorado State University showed incidences of it in dogs varied in different parts of the country. In that study we looked at two types of canine lymphoma: B-cell, which is the most commonly diagnosed; and T-zone, which is far less common. What we found is that there

are more B-cell lymphomas in some areas of the country than T-zone lymphomas, and vice versa. That leads us to think there are environmental triggers that cause these different types of lymphomas to occur. We don't yet know what those triggers are."

Genetically, the canine at-risk picture is a bit clearer. "When we talk about breed-specific risk in dogs," says Dr. Ruple, "we're really using breed as shorthand for genetic predisposition. We can look at breeds relative to each other, control for breed-population size, and see there's a vast difference in the number of lymphomas happening in certain breeds. We see the same thing in human populations, say in certain family members that have inherited a gene mutation for cancer, such as for breast cancer."

Adds Dr. Promislow, "Some high-risk breeds are 10-times more likely to get lymphoma than some low-risk breeds. That's a huge variation. We don't know why. That's what we're trying to find out." According to him, higher risk breeds include American Staffordshire Terriers, Beagles, Bernese Mountain Dogs, Boxers, Doberman Pinschers, Rottweilers, Shih Tzus, and Welsh Corgis. Lower risk breeds include Bulldogs, Collies, Dalmatians, Dachshunds, Poodles, Pugs, and Spaniels.

"Cancers happen when cell mutations occur," says Dr. Promislow. "Those mutations increase the ability of cells to divide and avoid being recognized by our immune system. That allows them to metastasize (spread)--and that gets us in trouble. We don't know what's different between the high-risk and low-risk breeds. We're asking, 'Are they more likely to get these mutations in the first place?'"

MAGIC MARKERS

The answer likely lies in genetic "markers," in this case, mutations. CHF's lymphoma study will use the University of Washington's highly accurate Duplex Sequencing technology, a breakthrough method for sequencing genomes, to find these markers.

"Our goal is to see if a blood-based biomarker can be developed to explain variation for lymphoma across dog breeds, and to predict risk," Dr. Promislow says. "We're focused on differences in mutations that set the stage for cancer, called 'precancerous mutations.' We'll measure the rate of early mutations in healthy dogs across ages and breeds. We're asking, 'Do higher-risk breeds have higher frequencies of precancerous mutations?'"

If the study can find a prognostic marker, or lead researchers to find one in the future, the benefits will be many. "If we develop a test that can recognize if your dog is at higher risk than normal for lymphoma due to this marker, we can't change his genetic risk," says Dr. Ruple. "But we can be proactive about testing him

frequently so we can diagnose lymphoma as soon as possible if and when it happens. That way, we can treat the cancer early, and he can live a longer life."

If a genetic marker is identified, it might be possible to reduce or eliminate the risk with selective breeding within at-risk breeds. "I'm optimistic about the possibility of eventually being able to breed out lymphoma," she says. "But we also need to examine dogs' environments to find what triggers the mutation to become cancer. Then we need to remove those triggers. This applies not just to dogs, but to humans."

She adds, "Our relationship with dogs has changed over time. They're not just living in our homes, they're sleeping in our beds. Their environmental exposures are far greater than ours. They're



Environment matters when it comes to healthy aging in dogs and in humans. Says Dr. Promislow, "Dogs live with us. They drink our tap water. They walk, sleep, and play on our floors, on our furniture, and in our yards. What we learn about them is likely to apply to us."

walking barefoot on the cleaning products we use, the lawn and garden products we apply, and the pesticides we spray, then licking their paws. They're lying, playing, licking, and sniffing in areas on which these chemicals are applied. They're our canaries in the coal mine: We're going to see environmental impacts in dogs before we see them in humans. Our dogs are going to help us find environmental factors that are causing cancers in them and us."

"The CHF lymphoma study sets the stage to look at mutations in dogs on a much broader scale within the Dog Aging Project," says Dr. Promislow. "It could potentially identify environmental factors associated

with higher mutation risk, or help lay the foundation for identifying them in the future."

Back to the earlier question that launched Dr. Promislow's focus on dogs and aging: Does size matter? "This mutation search has nothing to do with breed size," he says. "Lymphoma can happen in small-breed dogs as well as large-breed dogs. But there are some cancers, such as osteosarcoma (a bone cancer), that are more common in large-breed dogs. In general, larger breeds get more cancer than smaller ones. Further research may help us learn why."

Adds Dr. Ruple, "The entire Dog Aging Project should have many benefits for dogs and humans. Our lymphoma study, and DAP, are about helping both species live longer, healthier lives. That's the ultimate goal."

How long will the Dog Aging Project go on?

"I think of it as a 'forever project,' says Dr. Promislow. "The dogs we're studying now won't be around in 20 years. I'll be retired in 20 years. But I hope what we've built will still be here, and researchers can take advantage of the infrastructure to learn more about all kinds of things in dogs—and people."