PURINA PRO PLAN

GOLDEN RETRIEVER Update

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NEURONAL CEROID LIPOFUSCINOSIS

Rare Recessive Degenerative Neurological Disease Comes to Light

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GOLDEN RETRIEVER BREEDERS CAN USE DNA TEST

TO REDUCE THE RISK OF NCL

When a 1 1/2-year-old field-bred female Golden Retriever named "Lexi" (Turbo's Timeless Treasure) was confirmed to have the rare recessive degenerative neurological disease, neuronal ceroid lipofuscinosis (NCL), in the fall of 2018, it began a scurry of submissions of DNA from Golden Retrievers throughout the country for genetic testing.

It was heartbreaking to Turbo Golden Retriever breeders Ron and Pat Rubrecht of Land O'Lakes, Florida, to learn that a dog from a litter they bred had NCL — a disease they had never heard about — and one they sold to a field-trial home. The cause of Lexi's restlessness, anxiety, pacing, declining motor skills, bumping into things at home, and snapping at flies that weren't there was due to this lysosomal storage disease.

Essentially a failure of the lysosome system that recycles amino acids, or proteins, in cells, NCL occurs in Golden Retrievers due to a mutation in the *CLN5* gene. This results

in the accumulation of ceroid lipofuscin — an unusual material that glows fluorescent yellow when examined under a microscope — in the brain, retina and other tissues. Affected dogs develop normally until the storage material builds up and interferes with functioning. Golden Retrievers typically show signs around 1 to 2 years of age.

Fortunately, DNA testing is available that allows Golden Retriever breeders to determine their dog's genetic status and avoid producing puppies destined to develop NCL. This autosomal recessive condition occurs when the sire and dam are carriers and produce a litter in which an average of 25 percent of puppies are affected. As predicted by the odds, Lexi's littermate sister, "Gracie," also had NCL.

It was imperative to Ron Rubrecht to raise awareness about NCL and the genetic test developed at the University of Missouri. He reached out to the owners of puppies he bred that were potentially affected. Topbrass Golden Retriever breeder Jackie Mertens, of Palm Bay, Florida, the breeder of Lexi's sire, "Laddie" (Topbrass Lad Of The Lakes MH WCX), helped get the word out to the field community.

Topbrass Golden Retriever breeder Jackie Mertens, shown with Topbrass Chariot of Fire WCX, helped get the word out to the field community about the importance of testing dogs for NCL. Although there have been carriers in her 10 generations of field Golden Retrievers, she considers herself fortunate to not have produced any affected dogs.

More than 400 dogs were tested quickly when the news came out. DNA was sent for processing from living dogs and also dogs passed away that had been stored at the Canine Health Information Center (CHIC). Understanding the tangled web of the disease's origin in particular bloodlines was possible, providing that DNA was available to screen dogs.

Mertens learned about NCL carriers weaving through the Topbrass bloodline. Laddie's littermate sister, "Libby" (Topbrass Liberty Belle III CD MH QA2 WCX VCX CCA OD), was a carrier. In Libby's first breeding, sired by "Boomer" (FC AFC FTCH AFTCH CAN OTCH TNT's Explosion AM UD FDHF OS CAN FDHF OBHF), there were two carriers. Particularly concerning was whether "Hawk" (AFC Topbrass Hawk's Blackhawk OS), an NCL carrier, had produced affected dogs in three recent litters. The Outstanding Sire and second all-time top Golden Retriever derby dog has many desirable working traits.

"We were fortunate not to find any affected dogs," Mertens says. "Hawk has sired about 15 litters, and none has produced affected puppies. It is pure luck that the bitches

PROGRESSIVE SIGNS OF NCL IN GOLDEN RETRIEVERS

- **15 Months of Age:** Early neurological signs include loss of coordination causing dogs to bump into objects, difficulty climbing stairs, anxiety, agitation, and long periods of constant pacing and circling. Affected dogs may lose the ability to recognize or respond to previously learned commands and behaviors, have mild seizure-like activity, such as fly biting, or snapping at flies that are not there, and gum smacking.
- **18 Months of Age:** Seizure activity becomes progressively more severe and begins to involve the whole body with some episodes lasting up to an hour. Visual impairment in bright and dim light becomes profound. In later stages, affected dogs may become aggressive toward people.

Source: Gilliam D, Kolicheski A, Johnson GS, et al. Golden Retriever Dogs with Neuronal Ceroid Lipofuscinosis Have a Two-Base-Pair Deletion and Frameshift in *CLN5*. *Molecular Genetics and Metabolism*. 2015;115:101-109.

that have been bred to him were all clear of the NCL genetic defect."

Traveling back in her breeding, Mertens now knows that Libby and Laddie's dam and Lexi's granddam, "Paws" (AFC Topbrass Pawsability OD FDHF), who passed away in 2013 at the age of 14 ½, was a carrier. An Outstanding Dam, GRCA (Golden Retriever Club of America) Field Trial Hall of Fame inductee and a double-header winner, Paws and Mertens were inseparable. She also knows that Paws' sire, "Vinny" (FC Windbreaker's Premium Vintage), who died in 2000, was a carrier. Owner Pat DeNardo had Vinny's stored blood tested when she learned about NCL.

Mertens doesn't know whether Vinny's dam, Topbrass Tripp Of Deerwood OD, or maternal grandsire, "Cotton" (NAFC FC AFC Topbrass Cotton OS FDHF), were carriers. The only Golden Retriever to win the National Amateur Field Trial Championship, which he won in 1985, Cotton represented the third of 10 generations of Topbrass field Goldens.

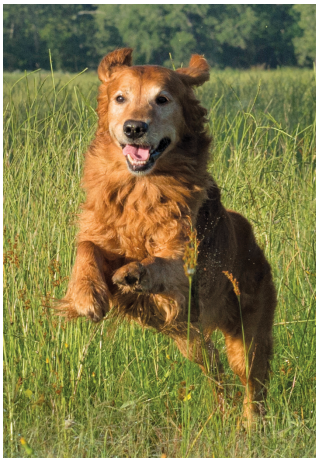
Topbrass Golden Retrievers are not the only bloodline affected by NCL. The mutation is found in several other field lines, as well as in the pedigrees of show-bred Golden Retrievers.

Taking a common-sense approach on how to stop producing puppies born with NCL, Mertens says, "The best advice is to test the sire and dam before you breed because you can breed a carrier, you just can't breed a carrier to a carrier. You don't want to throw out good genes or reduce genetic diversity."

FINDING NCL IN GOLDEN RETRIEVERS

Golden Retrievers are one of many breeds in which NCL gene mutations have been

RESPONSIBLE BREEDING TIED TO GENETIC TESTING FOR NCL IN GOLDEN RETRIEVERS



A DNA test for the *CLN5* mutation that causes neuronal ceroid lipofuscionosis (NCL) in Golden Retrievers is available through the Orthopedic Foundation for Animals. Having a recessive inheritance pattern, NCL occurs in dogs in which both the sire and dam are asymptomatic carriers. A carrier-to-carrier breeding produces progeny in which, on average, 25 percent are affected, 50 percent are carriers, and 25 percent are clear.

Through April 22, 2019, NCL genetic testing at the University of Missouri, including samples sent for research and DNA banked at the Canine Health Information Center, has been done on 3,190 Golden Retrievers. The majority, 3,010, were found to be clear. There were 153 carriers, and 27 dogs were affected.

"This is a low frequency for the overall Golden Retriever population," says Liz Hansen, project coordinator at the Animal Molecular Genetics Laboratory of the University of Missouri. "As some unfortunate breeders and owners know, this is a deadly and devastating disease that kills young dogs just entering the prime of their lives. This makes it very important to know the genetic status of dogs being bred so two carriers are not bred to each other, creating a risk for affected puppies."

Rhonda Hovan, research facilitator for the Golden Retriever Club of America, advises, "The use of DNA tests to reduce recessive disease genes should be done slowly and safely, recognizing that carriers very often have value as breeding dogs even when they carry serious diseases such as NCL. Our breed cannot withstand repeatedly wiping out disease genes in a short few generations, because all the generations that follow will be at risk for 'new' diseases driven to the surface by continually reducing genetic diversity.

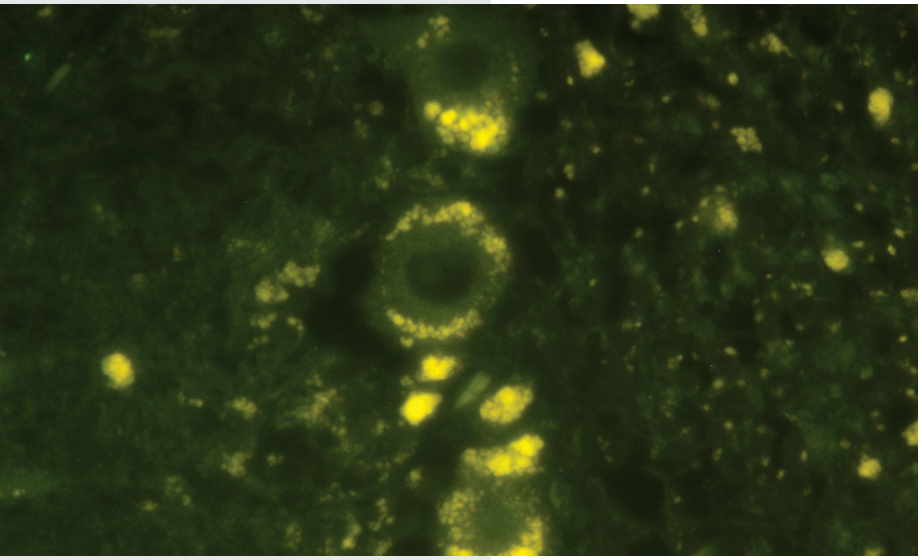
"Breeders should be careful to avoid too hastily purging carriers because rapidly reducing the breed's gene pool almost guarantees that the next genetic disease is right around the corner – and sometimes it's worse than the initial targeted disease."

discovered, though the disease results from different mutations among the breeds, occurs at different ages depending on the underlying mutation, and has different clinical presentations and different penetrance rates between the different forms of NCL. In most breeds, except Tibetan Terriers, Border Collies and American Bulldogs, it appears to be a rare disease, though it is likely that many cases of neurological disease in dogs are not recognized as potential NCLs

NCLs also occur in people and include the fatal Batten disease that affects children. Symptoms of NCLs in people include visual impairment, declining cognitive and motor functions, seizures, brain atrophy, and early death.[1] As many as 13 NCL gene mutations are recognized. Among animal species, NCLs are most frequently reported in dogs, though one or more cases have been reported in cats, sheep, cattle, ducks, ferrets, goats, horses, monkeys, parrots, and pigs.[1]

"Regarding similarities among the breeds, all of the NCLs occur because of mutations in genes involved in the necessary breakdown of protein that have accumulated damage over time," says Martin L. Katz, PhD, director of the Neurodegenerative Diseases Research Laboratory at the University of Missouri. "These damaged proteins cannot be degraded and accumulate in the cell to such a high degree that they are likely to impair cell function, particularly the function of nerves."

The genetic groundwork that led to the DNA test for Golden Retriever NCL was done in Dr. Gary Johnson's laboratory at the University



A disease affecting the lysosomal system that recycles proteins in cells, NCL causes an accumulation of ceroid lipofuscin, an unusual material that glows fluorescent yellow when examined under a microscope. It appears in the brain, retina and other tissues of affected dogs.

of Missouri. It began in the summer of 2014 when a breeder submitted 18 DNA samples of three generations of a Golden Retriever family after producing two litters of nine puppies from the same healthy parents, in which four dogs from the first litter showed signs of a progressive neurological disease. The published study in which the disease-causing mutation was reported appeared in the June-July 2015 issue of *Molecular Genetics and Metabolism*.

"The parents had the same healthy dam, but each had a different sire," Dr. Katz says. "The four dogs from the first litter exhibited progressive neurological signs characteristic of NCL starting around 13 months of age. Anxiety, constant circling, tremors, aggression, ataxia (incoordination), seizures, and visual impairment were among the abnormalities that became apparent at 15 months of age or older."

Whole genome sequencing of the DNA from one affected dog led to the identification of a homozygous mutation in the *CLN5* gene that produced a frameshift and premature termination codon.[1] Genotyping was performed on the common granddam, maternal grandsire, sire and dam, clinically affected female and her littermate sister, three normal littermates, and all nine puppies from the second litter. The researchers also genotyped 1,062 Golden Retrievers randomly selected from archived DNA at CHIC and their own database, and 143 dogs representing 99 breeds other than Golden Retrievers.

BREEDING IMPLICATIONS FOR NEURONAL CEROID LIPOFUSCINOSIS IN GOLDEN RETRIEVERS

	Clear Male	**Carrier Male**	**Affected Male**
Clear Female	100 Percent Clear	50 Percent Carrier & 50 Percent Clear	100 Percent Carrier
Carrier Female	50 Percent Carrier & 50 Percent Clear	25 Percent Clear, 50 Percent Carrier & 25 Percent Affected	50 Percent Carrier & 50 Percent Affected
Affected Female	100 Percent Carrier	50 Percent Carrier & 50 Percent Affected	100 Percent Affected

Source: Interpreting Your DNA Results for Autosomal Recessive Diseases. Veterinary Genetic Services.

"The findings from these analyses indicate that a dog with a defect in both copies of a gene called *CLN5* is unable to make a protein that is important for maintaining normal functioning of the nervous system," Dr. Katz explains.

Two of the affected littermates from the three-generation family became progressively worse until they were euthanized at 30 to 31 months of age due to the severity of their disease. Two additional dogs from the same litter had similar progressive neurological signs. Postmortem evaluation of the brain and retinal tissues confirmed that the disorder was a form of NCL.

Veterinary neurologist Dennis O'Brien, DVM, PhD, the Chancellor's Chair in Comparative Neurology at the University of Missouri College of Veterinary Medicine, explains, "When a Golden Retriever inherits the *CLN5* gene mutation, there is a defect in a series of three DNA molecules called codons that code for individual amino acids. In this case, two of those DNA molecules are knocked out, causing a frameshift to the left and premature termination codon. As a result, when the two DNA molecules are deleted, the amino acid sequence is altered and the protein does not function as it should."

As the recycling center of cells, lysosomes break down proteins into amino acids that can be reused to build new proteins. "A new specific enzyme is required for each step in the breakdown of a complex protein into amino acids," Dr. O'Brien says. "A hereditary deficiency in one of those enzymes sets up a road block in the breakdown of the protein. The fragments of protein then build up in the lysosome, which is where the name lysosomal storage disease comes. Over time, either the buildup of the degraded protein or the lack of the recycled amino acids interferes within the function of the cells and progressive disease results."

Because lysosomal disease only affects the recycling of proteins, dogs with the disease initially produce all the protein they need and thus may develop normally for the first year or so. "It is only when enough storage material builds up in the cells to interfere with functions that the clinical signs become apparent," says Dr. O'Brien.

As the disease progresses, so does the severity of clinical signs. This is what happened to Lexi. Her condition became progressively worse until she was euthanized in November 2018. Her littermate sister, Gracie, also was euthanized.

Although NCL is a challenging disease for an owner or breeder to face, with the discovery of the gene mutation and development of the NCL genetic test for Golden Retrievers, selective breeding can ensure affected dogs are not produced. "The nice part is you'll never breed an affected dog if you use genetic testing," Mertens says. ■

[1] Gilliam D, Kolicheski A, Johnson GS, et al. Golden Retriever Dogs with Neuronal Ceroid Lipofuscinosis Have a Two-Base-Pair Deletion and Frameshift in *CLN5*. *Molecular Genetics and Metabolism*. 2015;115:101-109.

Purina appreciates the support of the Golden Retriever Club of America, particularly Rhonda Hovan, GRCA research facilitator, in helping to identify this topic for the *Golden Retriever Update*.

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