

Golden Retriever Health and Genetics Highlight: Neuronal Ceroid Lipofuscinosis in Golden Retrievers

Ann F. Hubbs DVM, PhD and Ron Rubrecht, Turbo Retrievers



Lexi, the Golden Retriever who brought NCL to the attention of GRCA and in doing so helped future generations of Goldens.

In the fall of 2018, a Golden Retriever breeder learned that neuronal ceroid lipofuscinosis (NCL) was causing a fatal inherited disease in Golden Retrievers. Most of us had never heard of NCL, but affected Golden Retrievers from popular bloodlines were diagnosed at the University of Minnesota and later confirmed by DNA testing at the University of Missouri College of Veterinary Medicine. The good news was that a DNA test was available. Ron Rubrecht brought us the information about NCL and indicated that the real hero in this matter was "Lexi," Turbo's Timeless Treasure, an affected Golden Retriever.

Lexi's Story

Lexi's trusting owner, Sarah Kruger, had purchased what was expected to be a healthy and talented field-bred Golden. In June 2018, Sarah contacted Ron and Pat Rubrecht, owners of Turbo Retrievers and Lexi's breeders, asking for advice regarding Lexi. Lexi had been with a professional field trainer since she was six months old (whelped 1/23/17). The trainer indicated that, after wintering in Texas and after her first heat cycle, Lexi's past proven abilities were declining. Ron and Pat contacted the trainer and discussed all conceivable possibilities, including his opinion that there was something wrong with her vision. Lexi returned home to Sarah a different dog; something was definitely wrong. She was restless and anxious, and seemed to be always pacing. Her motor skills had begun to decline and she was running

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into things in the house. She began snapping at flies that did not exist.

Sarah had Lexi examined by an ophthalmologist and her eyes were normal. Sarah wound up at the University of Minnesota and was seen by their neurologist. The neurologist did a brain MRI and behavior observations of Lexi, and found that the atrophy detected in her brain and the behaviors were consistent with NCL. After several discussions with the University of Minnesota neurologist, it became clear that this condition was from a mutated recessive gene. The neurologist indicated that there was no genetic test for this in Golden Retrievers. He stated that he could only verify the diagnosis after a necropsy on the dog. However, Sarah and Lexi were not ready for that. Evidently, the trademark observation of the waste material build-up in the nerve cells of the brain was a visible florescent material which could be observed under a microscope. In mid-September, with the NCL diagnosis in hand, Ron undertook the job of learning more about this disease.

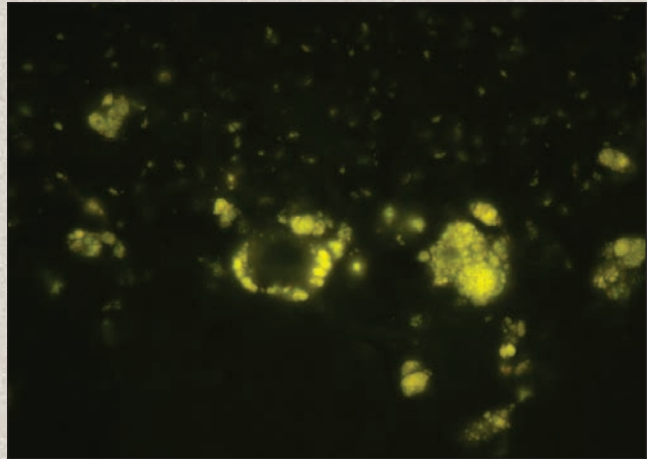
After a month of searching on the internet and discussions with several of the genetic testing labs in the country, Ron contacted the University of Missouri – Columbia, MO. It was as if they were expecting the call and knew the answer to almost every question posed about NCL. Further, they had a test for exactly what was described and suggested that a blood sample be submitted from Lexi to see if her condition matched the researched mutation which they had isolated. The test results confirmed that Lexi was affected with NCL.

Emotions ran high, as the confirmation of this horrible disease brought sorrow for Lexi's fate of a life that would be cut short, concern for her siblings and distress in the knowledge that her sire and dam were carriers from mainstream field lines. The other breeders close to these pedigree lines had already been notified and were working to understand the problem and the extent of its prevalence in our breed.

In early December 2018, tests of the parents confirmed their NCL carrier status. Ron and Pat Rubrecht would like to gratefully acknowledge the assistance, counseling, and participation by Jackie Mertens of Topbrass Retrievers, who was instrumental in swiftly obtaining the attention of the Golden Retriever field community. The Golden Retriever field community should also be commended for their prompt action to get some 400 dogs tested quickly after gaining this knowledge.

With this new knowledge in hand in mid-December 2018 and understanding the severity of this condition, the information was immediately released to the Golden Retriever public, so that everyone could be alerted before another affected puppy was placed into the hands of an unsuspecting and trusting puppy buyer. Now Ron and Pat are dealing with the fallout of this discovery. With the understanding of which Goldens were carriers, additional pups in a second litter were at risk, all with a 25% chance of being affected, and then other litters to consider.

Ron and Pat contacted the owners of potentially affected puppies. Coincidentally, the owner of one of Lexi's littermates had already contacted them about suspected behaviors. Based on the behaviors of Lexi's littermate, "Gracie" (Turbo's Amazing Grace), it was highly likely that she, too, was affected. During this time, Gracie's owner, having undergone



A fluorescence micrograph of the cerebellum from an affected dog. The cells across the middle with the most storage material are called Purkinje cells. Photo credit: University of Missouri.

a medical procedure, had entrusted Gracie's care to Ron and Pat while she recuperated. They strongly suspected Gracie of being affected after witnessing an episode of fly biting. During her stay with Ron and Pat, Gracie was tested and found to indeed be affected. This is not the news a breeder wants to tell any puppy owner. Gracie's owner, a trusting puppy buyer, Mary Ann Sheehan, was served a death sentence for her beloved companion. Gracie did not seem to be as severely affected as Lexi but during her almost two months with the breeder, Gracie's health declined rapidly as her disease progressed. Gracie had a reasonable quality of life until her seizures began. It was because of the seizures, sometimes two in one day, and the stroke-like conditions following the seizures, along with failing motor skills, that the kindest decision was to have Gracie euthanized. She had out-lived her sister, Lexi, by two months.

Ron and Pat consider Lexi and her owner, Sarah Kruger, the true heroes to whom all Golden Retriever owners owe a debt of gratitude. Without Sarah's determined search for a proper diagnosis for her beloved Lexi, we would not have had the trail of knowledge that led us to where we are today.

Lexi's Legacy

This new knowledge of NCL went viral, gaining attention worldwide. Even now, we do not know how widespread this condition is or which lines are affected. We do know that this is found in field and show lines and on multiple continents. From recent stories being shared with the breeder, this condition may very well have been around for decades and misdiagnosed as seizures and epilepsy.

Since learning of Lexi's carrier-to-carrier litter, Ron and Pat identified another carrier-on-carrier breeding between Lexi's sire and the sister to Lexi's dam. They are now dealing with the test results of the second litter. As of this writing, there is just one more dog to test in the second litter of 11 pups. The test results have confirmed three more unsuspecting owners must deal with the death sentence presented to them in just the past few weeks. Additionally, there is another breeder that has another confirmed carrier-on-carrier litter

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with three affected dogs in the litter of seven pups. Even tonight, as this document is about to go to press, we have a report of another carrier-on-carrier litter with 12 seven-month-old pups that are now at risk. Also tonight, we are hearing from a breeder about a litter from 1985 with three NCL-affected dogs from show lines. Ron and Pat have been touched by the support they have received from the affected families who have helped to increase awareness. Thank you.

To date, there have been numerous relieved breeders and owners with newborn pups on the ground, in gestation, and others already in puppy buyers' homes with normal lives. There are others who have bred to now known carriers or close to suspected lines. There are additional litters in the pre-symptomatic age still awaiting test results, and some people may still be unaware or in denial about NCL.

At this time, Optigen has transferred DNA in their possession from past testing that has just been received at the University of Missouri. This initial batch includes samples from field dogs and will help to map out the trail of the carrier genes back in the affected lines for about the past 20 years. At that point, we run out of DNA, but there are hints in the past of seizures and epilepsy which may be a clue. This DNA transfer and testing will provide the status of some prolific and highly successful sires of our current field lines. This will answer many questions and will help to clear many dogs by parentage rather than additional testing. These revelations

are expected in mid-February 2019, just shy of the publication deadline for this document.

It is the overwhelming hope of the breeders and the owners of these affected dogs that every Golden Retriever breeder take this seriously for all current breeding stock, so as to never allow another unsuspecting family to develop deep bonds with an affected puppy only to see it ripped from their hearts in the second year of its life. If you test and find your dog clear or that its mate is clear, that will bring a peace of mind that these breeders wish they'd had. While others in the past who knew did not divulge this information to the Golden Retriever population, there is no longer any excuse for such complacency.

Most breeders and even most veterinarians know very little about NCL. We all need to get up to speed on this one because it is a devastating disease. A new NCL In Golden Retrievers page has been set up on Facebook to provide a teamwork approach to spreading awareness. This page has been set up by concerned Golden Retriever owners and supported by a University of Missouri staff member. This site is being used to convey up-to-the-minute technical information, statistical data, and new test results. The revelations and disclosures are already too numerous to count. There are currently 1903 members of this page which was created just two weeks ago.

Below is a summary of what we know today.

Information About NCL from the Health and Genetics Committee

NCL is a neurologic disease caused by the accumulation of material (ceroid and/or lipofuscin) in a part of the cell known as the lysosome, that would normally serve to digest that material. Material accumulating in the lysosomes causes a large group of diseases called the lysosomal storage diseases. The lysosome is the garbage disposal of the cell and also plays many other roles and it does not function correctly in NCL (5). Today we know that there are many different types of NCL and different mutations in different genes are able to cause NCL. NCL can be diagnosed at necropsy (an autopsy of a dog) because of the fluorescent material that collects in the cells of affected dogs. It is the accumulation of material that should be removed that leads to the signs of NCL and eventually the death of affected dogs. If the DNA mutation causing a specific type of NCL is known, affected and carrier dogs can be identified by a DNA test. The signs of NCL usually develop between one and two years of age and include a lack of coordination, tremors, vision problems, behavior changes and seizures.

NCL was originally discovered in Golden Retrievers because of a single affected litter diagnosed at the University of Missouri and the results were published in 2015 (1). Three puppies in a single litter developed signs of neurologic disease, beginning at about 15 months of age. Researchers at the University of Missouri diagnosed NCL and a mutation in the **CLN5** gene. The Golden Retrievers affected with NCL were homozygous for a previously undescribed mutation in CLN5, a gene known to cause NCL when mutated in humans or bor-

der collies. What is truly amazing is that the researchers used whole exome sequencing, a technique that looks at all coded portions of the DNA in order to identify specific mutations that distinguish affected dogs from those without disease. It is relatively new, even in human medicine, and it is expensive (thousands of dollars per sample).

Golden Retriever NCL was identified as a recessive genetic disease due a CLN5 mutation not seen in any other breed of dog (1). Eleven Golden Retrievers closely related to the original three affected puppies were carriers. All carriers were clinically normal indicating that this is a recessive disease.

At the time of the 2015 publication, CLN5 was believed to be rare in the general population of Golden Retrievers (1). The researchers who originally identified the CLN5 mutation examined the frequency of that mutation in a Golden Retriever population. In 1062 Golden Retriever DNA samples randomly selected from the Golden Retriever DNA available at the University of Missouri, 8 had a single copy of the gene and one was homozygous/affected. Therefore, **the historical carrier rate for the CLN5 mutation in that sample of Golden Retrievers was 8 of 1062 or 0.75%.** The total percentage of carriers from combined clinical and random samples at the University of Missouri through October 2018 was 1.8% carriers.

Today, NCL is not rare in some very successful Golden Retrievers families. Purebred dog breeding is not random.

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A couple of successful dogs can cause a dramatic shift in the prevalence of a devastating disease very quickly. At the time DNA was collected for the published paper, the carrier rate for NCL outside of the original family was estimated to be 8 in 1062 (0.75%). With random mate selection, the odds of a second carrier being bred to a carrier would also be 8 in 1062 so the odds of that happening would be 0.7533% of 0.7533% or 0.000565% or about 5.7 in 100,000 litters being affected if one assumes a 100% conception rate. If breeding was random and that DNA represented the population, that data indicated that NCL would be a rare disease. It is important to remember that DNA available to researchers may not fully represent the Golden Retriever population and also that the frequency of specific disease genes can change with time. Whatever the cause, clearly the samples did not reflect the population frequency of this mutation and/or something has changed.

At the time this is written, the DNA test specific for Golden Retriever NCL has been well publicized and commercially available in the US for a few weeks and owners can post test results on the k9data website <http://www.k9data.com/>. We now have much more information than we had in 2015. The current numbers are highly influenced by the obvious fact that k9data is a voluntary database and owners of Goldens related to NCL-affected Goldens are doing more NCL testing than average owners. Today, k9data lists 412 clear, 40 carriers, and 9 affected or 9.7% carriers and 2% affected. Thus, affected Golden Retrievers are being identified by combined DNA testing and clinical disease and additional Goldens have been identified as carriers by DNA testing. While that data is heavily influenced by owners prioritizing the sampling of Golden Retrievers related to the ones affected with NCL and the obvious limitations of an open database, **we clearly have a problem with NCL today.**

The most likely cause of the apparent increase in the number of NCL cases is the tremendous success of some carriers and their use for breeding. Another factor may also have contributed. Breeders were aware of ichthyosis in some Goldens and a DNA test was available. It is possible that some sires that were ichthyosis normal/clear and have turned out to be carriers of NCL received increased use at stud because of their ichthyosis normal/clear status. However, unequivocally, some carriers were highly successful and breeders wanted their offspring. Some of the descendants of the highly successful NCL carriers were not only successful but some of them were also NCL carriers. Eventually a significant number of carriers were bred together and the result was an increase in the number of affected Golden Retrievers. As more and more genetic tests become available, we have to be careful, as much as possible, not to let new devastating conditions become common. Trying to maintain genetic diversity is so crucial, and so hard. That is where we are today.

DNA testing can prevent future cases of NCL. All known cases of Golden Retriever NCL in the U.S. have tested as homozygous/affected using the Golden Retriever NCL test developed at the University of Missouri (1). Genetic testing

for NCL based upon that technology is also available from other laboratories including some in other countries. **To prevent Golden Retriever NCL, carriers should only be bred to Goldens tested as normal/clear. This can be accomplished by testing both the sire and dam or being certain that at least one of them is NCL normal/clear.**

We can learn a great deal from experience gained from DNA testing in other breeds using DNA testing to control other devastating recessive lysosomal storage diseases and from the experience of Golden Retriever breeders using the test for prcd-PRA, a DNA test that has been available for more than a decade. To help us learn from those experiences, we are reprinting an article previously published in *Today's Breeder*. That article tells the story of how Portuguese Water Dog breeders avoided breeding carriers of a devastating lysosomal storage disease GM1-gangliosidosis. That resulted in two things, 1) a virtual elimination of Portuguese Water dogs affected with GM1-gangliosidosis, and 2) an increase in blind Portuguese Water Dogs. The cause of the blindness was *prcd-PRA*, a genetic disease often carried by the major bloodline with lower numbers of GM1-gangliosidosis carriers. The lesson is particularly important today, with more than 700 inherited diseases and traits currently described in dogs (2). Undoubtedly many more genetic diseases will be described in the future using the same technology that allowed the discovery of Golden Retriever NCL.

One of the greatest protections against the emergence of new genetic diseases in dogs is maintaining genetic diversity within each breed (3). For that reason, the best protection against recessive genetic diseases is identifying carriers of devastating diseases like NCL and being sure that if they are bred, their mates are normal/clear. Cheek swab testing of puppies from litters produced by carriers can help breeders identify carriers within a litter so that preferences are made for the normal/clear puppies when selecting homes where they may potentially be bred in the future. The idea is to maintain genetic diversity and quality by allowing quality carriers to be bred while preventing affected puppies. A recent study indicates that DNA testing can accomplish tremendous decreases in the prevalence of deleterious genes. That study showed a greater than 90% reduction in genes for eight genetic diseases in dogs born 8-10 years after the release of a DNA test for those diseases (2).

For additional information on NCL in Golden Retriever, the Comparative Neurology Program at the University of Missouri College of Veterinary Medicine has developed a website with additional information. The link to that information is at: http://www.caninegeneticdiseases.net/GoldenNCL/?fbclid=IwAR0v4gFmnONLvuFR3_YKENOjttwc8tWultki4LN7r0dHdMa7LdvJTWEv3QU

Summary:

- 1) NCL is a devastating progressive neurologic disease that is fatal in affected (homozygous) individuals
- 2) The University of Missouri developed a test for NCL in Golden Retrievers that correlates with clinically affected dogs and carriers
- 3) The basis for that test is published in the peer-reviewed

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scientific literature

- 4) NCL is believed to be rare in the general Golden Retriever population
- 5) NCL is common in some Golden Retriever populations
- 6) A test specific for Golden Retriever NCL is available
- 7) Golden Retriever NCL **carriers** should only be bred to Goldens tested as **normal/clear**.

Given the severity of NCL, everyone should consider if they want to take the chance of producing a puppy with NCL. No one wants to be in that position. For that reason, the H&G recommends:

Prior to breeding, at least one parent of each prospective litter should be confirmed as normal/clear for NCL by DNA testing or pedigree. Optimally, the NCL status of both parents should be known since this helps identify litters that are normal/clear by parentage. NCL carriers should only be bred to Goldens tested as NCL normal/clear.

Laboratories Currently Advertising Testing in the US

1) **University of Missouri/Orthopedic Foundation for Animals** has a test specific for Golden Retriever NCL and is based upon DNA you can collect from a cheek swab <https://www.ofa.org/diseases/dna-tested-diseases/neuronal-ceroid-lipofuscinosis> .

2) **The Embark DNA panel tests for 165 health conditions, some that are, and some that are not, known to affect Golden Retrievers.** This technology is the technology often used to identify the breeds behind dogs of unknown breeding (e.g. the relationship between a crossbred dog and specific breeds) and the genes associated with canine disease in published reports. Like the OFA test, an oral swab is used as the source of the DNA. **The Golden Retriever NCL variant is among the many health conditions included in the panel** <https://embarkvet.com/health-list/> . Due to the large number of health conditions included in the results of DNA test panels and because the predictability of associated clinical disease is not known for some of these in Golden Retrievers, many have emphasized the importance of consultation with a knowledgeable attending veterinarian regarding some of the health conditions included in large DNA panels (4). If using this method to test, it is important to remember that all people and all dogs are believed to carry some recessive genetic diseases (3).



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