



Epilepsy Gene

H U N T E R S

His seizures came in the middle of the night, long after the family had gone to bed. In the beginning “Preston” didn’t lose consciousness, but he foamed at the mouth, his lips quivered, and one front leg twitched uncontrollably. The focal seizures started when the fun-loving, full-of-life English Springer Spaniel was about 2 ½ years old, only weeks before the first, and subsequently last, litter he sired would be whelped.

Though she knew little about seizures, his owner, Georgian English Springer Spaniel breeder Karen Foster of Decatur, Ga., had “a sinking feeling and intuitive sense that things would never be the same.”

Five seemingly healthy puppies were born December 9, 1996, presenting what usually was great joy and happiness, but Foster wondered whether she should put the litter to sleep. “I was absolutely devastated,” she says.

Telltale signs had cropped up in her breeding program two years earlier when the puppies’ uncle, “Weaver,” a littermate to “Baker,” the dam of the litter, first had seizures. Weaver also was a half brother to “Kendal,” Preston’s dam. Foster had bred and co-owned Weaver, who lived with her good friend,



“Preston” was an English Springer Spaniel who experienced cluster seizures and died at age 5. Clockwise from top: Preston as an 8-week-old puppy. Preston, left, photographed with his three-quarter uncle, “Milton,” and his dam, “Kendal.” Breeder-owner-handler Karen Foster poses with Preston after he achieved Highest Scoring Dog in Trial at an Atlanta specialty in 1999.



the co-owner. At the time “I had heard of seizures in springers, but I thought Weaver’s epilepsy was an anomaly,” Foster says.

Sadly, despite efforts to treat Weaver’s epilepsy, he died during a seizure in 1994, a few weeks before Preston was born. Weaver was not yet 3

years old. “When Weaver died, I had bad feelings about what this might mean for my bloodline,” says Foster.

Friends, including Weaver’s owner, stood by Foster, still wanting Preston’s puppies. Her veterinarian advised her, “If they were not meant to be here, they wouldn’t be.” Two puppies went to

friends, and three went to other people along with a full disclosure about Preston's and Weaver's epilepsy. Foster would not allow any to be bred.

By now, Preston, the sire of the litter, was having grand mal seizures about every two weeks. These, too, came in the night. The English Springer would fall on his side, begin thrashing, foaming at the mouth, totally unconscious. "Two or three minutes seemed like two or three hours," Foster says.

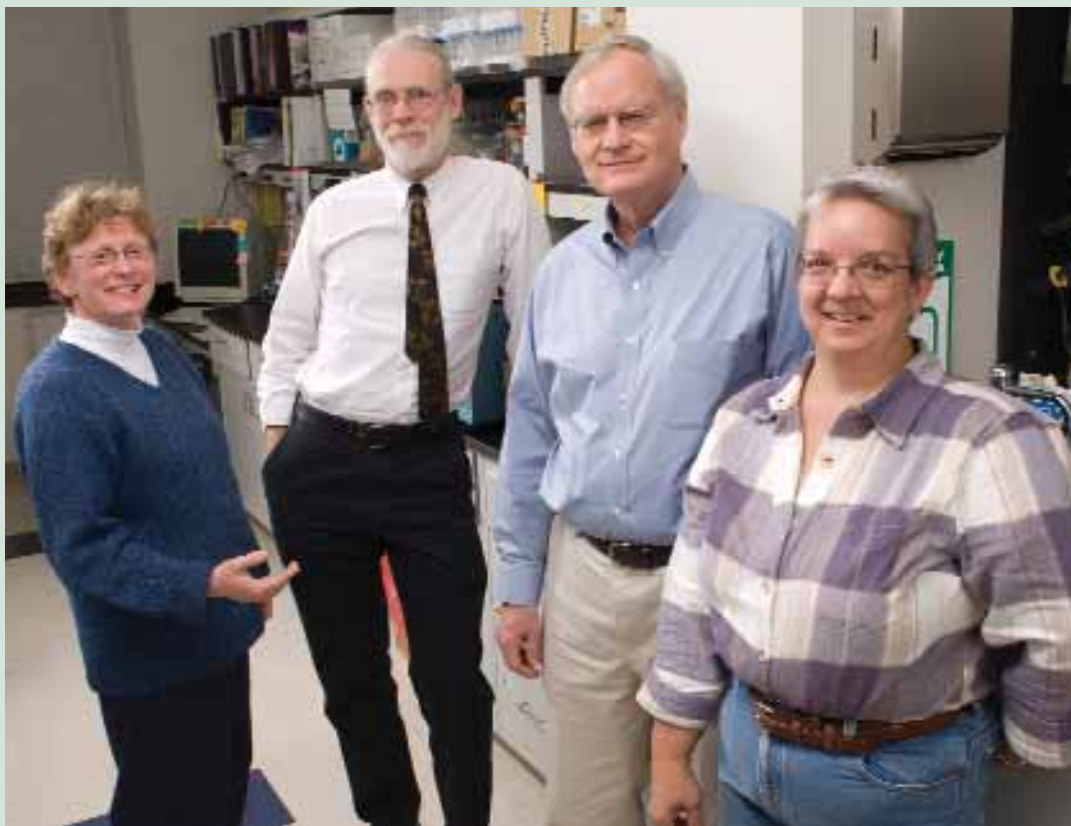
Then it worsened. "Preston went from having a single seizure to having clusters of seizures," says Foster. Four months after the seizures began, Preston had an episode of 30 seizures over 24 hours.

Though her veterinarian was only 35 minutes away, Foster had to first drive her beloved springer to a closer veterinarian for a shot of valium to quell the seizures so they could safely reach the regular veterinarian. Before, Foster had not wanted to medicate Preston for seizures; doing so would mean conceding that her dog truly had epilepsy. Now, there was no choice.

The veterinarian prescribed Phenobarbital right away. Potassium bromide, a less common treatment for canine epilepsy in 1997, would come 18 months later after Preston's liver began to show signs of damage from the Phenobarbital. The veterinary staff monitored Preston's seizures throughout the day. He was put on intravenous fluids. By nightfall, the veterinarian sent the dog home with Foster, who stayed by his side all night as the seizures continued.

Finally it was over. "It was hard on him," says Foster. "It took him three days to figure out who he was, where he was, and who I was. He had forgotten housetraining, and the physical stress had made him stiff and sore. After about one week, he was a regular dog again."

Preston's seizures waned after that first cluster, but he would have many seizures, including clusters, for the rest of his life. He was one of four springers who lived with Foster. A passionate breeder and competitor who had shown English Springers since she was a teenager, Foster helped Preston earn a Companion Dog title in obedience trials and a Working Dog certificate for proving his hunting



Researchers at the University of Missouri who are members of the Canine Epilepsy Research Consortium are, from left, Dr. Joan Coates, Dr. Dennis O'Brien, Dr. Gary Johnson, and Liz Hansen.

instincts. She tried tracking with him, but he didn't have the necessary concentration.

All in all, between the seizures, Preston "lived a great life. He loved everyone and was very upbeat," Foster says.

Preston died in 1999 at 5 years of age. As it turned out, only one of Preston's puppies had seizures. "Tucker" developed seizures when he was 11 months old and died when he was 2 ½ years old, six weeks before his sire. Like his sire, Tucker had experienced cluster seizures. Of the other four puppies sired by Preston, three are now 11 years old, and one died from unrelated causes.

Foster stopped breeding English Springer Spaniels after Preston died. "I had nowhere to go with these dogs," she says. "They were all closely related. I felt epilepsy had beaten Preston and me, and that was not acceptable."

The year before Preston died, Foster became deeply involved in the new epilepsy project supported by the breed's parent club, the English Springer Spaniel Field Trial Association (ESSFTA),

and the ESSFTA Foundation. She and a handful of other springer fanciers began recruiting blood samples from breeders at field trials and specialties. She discovered that "many people had a hard time believing we had dogs with epilepsy. Epilepsy is an ugly, frightening disease. Some people absolutely did not want to acknowledge it."

A Complex Disease

Since those early efforts, nearly 4,000 blood samples have been collected, making the English Springer Spaniel DNA bank the largest among all AKC breeds. Founded in 1996, the ESSFTA Foundation identified health priorities in the breed; epilepsy, the eye disorder progressive retinal atrophy (PRA), and the sugar-processing disease phosphofructokinase (PFK) deficiency were the top three concerns. Importantly, the Foundation had begun a DNA bank that would provide quality samples of extended families.

Discoveries of the gene mutations causing PRA and PFK led to genetic tests for both conditions.

Meanwhile, epilepsy proved to be a more complex, more difficult disease to understand. The ESSFTA Foundation raised \$50,000 to support ongoing epilepsy research, funding that was matched by the AKC Canine Health Foundation.

The first AKC Canine Health Foundation grant to study epilepsy in English Springer Spaniels and Standard Schnauzers went to Gary Johnson, D.V.M., Ph.D., associate professor at the University of Missouri College of Veterinary Medicine. A molecular geneticist, Johnson had the insight to begin collecting samples from members of canine epilepsy families so that their DNA would be available when powerful new mapping technology became available. Like the English Springer community, Standard Schnauzer breeders had gathered blood samples from an extended family affected by epilepsy. They, too, wanted to learn the inheritance pattern.

About the same time that Johnson and his colleagues at the University of Missouri began studying epilepsy in English Springer Spaniels and Standard Schnauzers, a University of Minnesota College of Veterinary Medicine team led by Edward “Ned” Patterson, D.V.M., Ph.D., DACVIM, assistant professor of medicine, genetics and epilepsy, began a similar study of epilepsy in English Springer Spaniels and Vizslas. Both groups were seeking the genetic cause of epilepsy; both realized the complexity of the disease and that potentially the pattern of inheritance would vary considerably from breed to breed. Thus began the Canine Epilepsy Research Consortium in 1999.

The consortium consists of an unofficial group of investigators, including veterinary and human clinicians, neurologists and geneticists, devoted to unscrambling the epileptic puzzle and helping breeders to better understand the disease and ultimately reduce the disease incidence in their dogs. The members share samples, data and pull resources to help advance the research. They also collaborate on research papers. The consortium includes research teams at the University of Missouri, the University of Minnesota and Florida State University and internationally at the Hospital

for Sick Children in Toronto, Canada, and the University of Helsinki in Finland.

Altogether more than 100 breeds of dogs are being studied at the different institutions, and funding is supported by parent clubs, the AKC Canine Health Foundation, the National Institutes of Health, the Canadian Institutes of Health Research, the European Union and the Academy of Finland. These experts estimate that from 1 percent to 5 percent of dogs have repeated seizures in their lifetime. Statistics related to the prevalence of epilepsy in specific breeds are often unreliable, says Dennis O’Brien, D.V.M., Ph.D., DACVIM, professor of neurology at the University of Missouri College of Veterinary Medicine.

“I do not want to point fingers at specific breeds because there are no good statistics on the incidence of epilepsy in the general population,” says O’Brien. “The breeds in which incidence has been reported are the ones that are actively working to eliminate the disease, but that does not mean the disease may not be more common in other breeds where the problem is being ignored.”

Largely, the researchers are studying idiopathic epilepsy, although some research has focused on rare, progressive forms of epilepsy. For example, the University of Missouri researchers successfully identified the genes responsible for neuronal ceroid lipofuscinosis (NCL) in English Setters, Dachshunds and American Bulldogs. Epilepsy occurs in the end-stage of this autosomal recessive neurological disease in which both parents must carry a copy of the gene mutation for offspring to be affected. NCL in dogs is similar to Batten disease in children.

Pediatric neurologist Berge Minassian, M.D., and his then postdoctoral fellow Hannes Lohi, Ph.D., at the Hospital for Sick Children in Toronto discovered the gene mutation causing progressive myoclonic epilepsy (PME) in Miniature Wire-haired Dachshunds, a condition affecting more than 5 percent of the breed. The gene mutation causing this autosomal recessive form of canine epilepsy is the same as that causing Lafora disease, the most severe teenage-onset human epilepsy. Here, too, a

dog must inherit a mutated copy of the gene from both its dam and sire to develop PME.

While NCL and PME are examples of progressive epileptic conditions with identifiable signs enabling diagnosis, idiopathic epilepsy is a disease of exclusions. Foster simplifies the definition. “A seizure is a symptom of something. It’s idiopathic epilepsy when you can’t find something else,” she says.

“The biggest challenge in idiopathic epilepsy,” explains Patterson, “is that it is a diagnosis that comes from excluding other causes of seizures such as a brain tumor, brain infection, metabolic disease or an abnormally formed brain. In most cases of idiopathic epilepsy about 66 percent of dogs have their seizure onset from 1 to 3 years of age.”

Idiopathic epilepsy is most likely caused by a biochemical defect in the brain cells or in the brain cell environment. An affected dog has normal physical, neurological and laboratory examinations during intervals between seizures. Confirming the cause of epilepsy is critical to successful treatment.

“When collecting information, we are trying to answer two questions: Is this really a seizure, and is there any underlying, non-genetic cause,” O’Brien says. “The length and character of the episode and the dog’s behavior before and after the episode all contribute to determining whether the spell is a seizure or something else, such as a dizzy spell or fainting. How a dog behaves between episodes and his medical history help us to determine whether there may be an underlying cause.”

Only 10 years ago, researchers believed that dogs with idiopathic epilepsy experienced generalized seizures exclusively. “It is now increasingly evident that many dogs with idiopathic epilepsy have focal-onset seizures in which only a part of the body is affected at first,” Patterson notes. “These focal seizures may stay focal or may turn into a generalized seizure.”

“Focal seizures arise in a localized area of the brain,” O’Brien explains. “As a result, the seizure is limited in nature. It could be twitching of one side of the face or body or simply the dog freezing and staring off into space. A generalized-

Quest Continues to Find Genetic Marker for Epilepsy in Belgian Tervuren

More than two decades ago, members of the American Belgian Tervuren Club (ABTC) realized that epilepsy had become a health problem in the breed. Affected dogs were generally around 3 years of age, and both males and females suffered from seizures.

The club organized an extensive survey on seizures and sent it to all members. The random sample yielded data on 997 Belgian Tervuren. Potentially, the information would lend credibility to the estimated frequency of seizures, and thus the occurrence of epilepsy, in the breed.

Unfortunately, good intentions fell short, and the surveys remained boxed up for many years, when no investigators could be found to analyze the data.

Eventually, the parent club contacted Thomas Famula, Ph.D., and Anita Oberbauer, Ph.D., both professors of animal science at the University of California-Davis, who agreed to have a look at the data.

Oberbauer, who is chairwoman of the ABTC Health Education Committee, already had a passion for Belgian Tervuren as a breeder and competitor in conformation, agility, herding, obedience and tracking. Though the survey was conducted many years before she became involved in the parent club, she says, "The goal behind the ABTC survey was to provide breeders with information about the hereditary of epileptic seizures."

Famula and Oberbauer presented their findings from the survey in 1996. Sadly, the analysis indicated that 17 percent of the breed is affected with epilepsy. The heritability range was reported to be 0.65 to 0.88, meaning that offspring of an affected dog have an extremely high risk of being affected.

"Heritability is a value that ranges from 0 to 1 and tells us the risk of disease for a dog if one, or both, of its parents have the disease," Famula explains. "In the case of Belgian Tervuren, the good news is that the high heritability means that a dog with no close relatives affected by the disease is at a very low risk of disease."

"Heritability estimates provide a basis to determine if selective breeding can help to reduce the disease," Oberbauer says. "The high heritability told us that significant progress could be made to reduce the incidence of seizures merely by practicing judicious breeding. Breeders could selectively not breed affected dogs or their relatives to help reduce the occurrence of epilepsy."

Offspring produced by non-epileptic dams and sires have a 0.99 percent of never suffering from a seizure compared with 0.58 percent for offspring produced by dams and sires who each had one seizure. The researchers also learned that the average age of onset is around 38 months of age but that 21 percent of Tervuren have a first seizure before age 2.

The investigators began to turn their attention to unraveling the genetic cause of epilepsy in Belgian Tervuren. They collected buccal swab samples and started building data from extended families of affected dogs and their affected and non-affected relatives. They recently conducted a genome-wide scan using microsatellite genotyping to study the relationship between genes and the seizure phenotype.

"Our objective is to identify through genetic markers the specific chromosomal region that is linked to the expression of seizures," says Oberbauer. "We have just completed the full-scale genome association using microsatellite markers, and the data are being analyzed."



Dr. Anita Oberbauer is not only a geneticist who is studying epilepsy in Belgian Tervuren, she also is an enthusiast who breeds and competes in several sports with her own dogs.

The findings support that epilepsy in Belgian Tervuren, as in many other breeds, is polygenic with a possible single locus of large effect. "This means that although many genes may affect the expression of the trait, a major gene is likely to play an important, though not exclusive, role in the inheritance of epilepsy," Oberbauer says. "The theory is that the allele combination at one locus is highly influential in whether the trait will be expressed."

"One school of thought about epilepsy is that the expression is threshold-regulated. If the genes combine to surpass a given threshold, then the animal will not experience seizures. Yet one gene within that combination of genes can significantly affect whether the threshold is surpassed, although the perfect combination of other genes may also permit surpassing the threshold limit."

Meanwhile, shortly after Oberbauer and Famula began investigating epilepsy in Belgian Tervuren, they began looking at the disease in a closely related breed, Belgian Sheepdogs. Today, the circle has expanded to include Poodles, Giant Schnauzers and English Mastiffs, all breeds chosen based on interest from

parent clubs or variation in gene pool size and disease characteristics.

"Ultimately, information on what chromosomal region is associated in one breed may lend clues to the genetics behind seizures in another breed," Oberbauer says.

Though the exact genetic cause of epilepsy in Belgian Tervuren remains a mystery, the number of dogs classified as epileptic has begun to decrease. Oberbauer credits breeders who are using selective breeding to help reduce the disease. "The knowledge that epilepsy is genetically controlled has helped breeders to rethink breedings and the risk association with particular combinations," she says. ■

What To Do When Your Dog Has Seizures

Owners of dogs that suffer from epilepsy often describe seizures as heartbreaking, partly because they do not know what to do to help their dog. Here are some tips from experts on what to do if your dog has seizures:

- Any dog that has had even one single seizure should be seen by a veterinarian. Dogs that have seizures more than once every six to eight weeks, even shorter time spans for some large breeds, should be treated. Seizures that occur in clusters or last more than five minutes also are criteria for treatment. A dog that is having seizures more than every six to eight weeks and is left untreated is likely to eventually have life-threatening seizures that last more than five minutes.
- The main responsibility of an owner during a seizure is to keep the dog physically safe, away from stairs or water.
- Do not attempt to open the dog's mouth during a seizure. Dogs will not swallow their tongue, and you could be easily bitten.
- Seizures become an emergency if a single seizure continues longer than five minutes, if three or more seizures occur in 12 hours, or if a dog does not recover between seizures. A dog should be taken to a veterinarian or emergency clinic as quickly as possible for emergency treatment. In some instances, a dog could be in a life-threatening state and could become hyperthermic secondary to the seizure. A veterinarian can give medication to help stop the seizure.
- After a seizure, an owner should keep a dog confined to a safe area so he can't wander off or into something that could cause harm.
- Owners should work closely with their veterinarian to find the right medication dosage and be sure to have recommended blood work to check drug levels and liver function on a regular basis.

Source: Edward "Ned" Patterson, D.V.M., Ph.D., DACVIM, University of Minnesota and other veterinarians.

seizure affects the entire brain at once. When focal seizures progress to generalized seizures, we refer to them as generalized seizures with focal onset.

"Brain cells are called neurons, and they are excitable. They communicate that excitement to other neurons via electrical and chemical signals. To balance out that excitement, some neurons are inhibitory or tell the next neuron down the line to settle down and not get so excited. Seizures occur when too many neurons become excitable all at once. You can think of it as an electrical storm in the brain where this excitation takes over and creates uncontrollable firing of neurons that we see clinically as a seizure."

Using SNP Chip Analysis

When canine epilepsy research began, the canine genome was not yet sequenced. Researchers relied on linkage analysis to identify markers near gene mutations. Now, research has fast-forwarded, and it is significantly easier to find mutated genes

once a chromosomal area likely to contain the gene has been identified. The process involves conducting whole genome scans using SNP (single nucleotide polymorphisms) chips to identify specific gene sequence variants. Although canine chromosomes from different dogs are more than 99 percent identical, they still vary at millions of sites. The majority of differences are SNP letter changes. Although some SNPs have functional effects that alter the biology of an animal, the majority of SNPs have no biological significance but can be used as markers to identify the chromosomal region carrying a mutation.

SNP chip analysis is being used by the University of Missouri team to analyze the DNA of an extended family of Australian Shepherds in which epilepsy heavily dots their pedigree. The actual SNP chip processing was done at the University of Helsinki, and the data currently is being analyzed.

"We tried unsuccessfully to map epilepsy by linkage analysis with a microsatellite-based genetic map in Australian Shepherds and Chinooks,"

Johnson says. "The Australian Shepherd DNA appears ideal for mapping epilepsy loci by allele association with canine SNP chips."

The Australian Shepherd community realized in 1993 that epilepsy was a potential health concern, and today, few breeders have bloodlines free of the disease. A grass-roots effort helped to promote the sharing of pedigree information about affected dogs and their non-affected relatives, epilepsy education, and research participation through the contribution of DNA. More than 1,600 blood samples were collected, representing a huge extended family.

Though a single autosomal recessive gene is suspected to be the gene mutation, investigators won't know definitely until the SNP chip analysis is completed. If epilepsy turns out to be a polygenic condition, it could be more complicated to identify the mutation. The goal is to develop a direct DNA test that will allow breeders to identify which Aussies have the potential to develop the disease and which healthy dogs are carriers. Only about 6,000 Australian Shepherds are registered each year with the AKC. Hopefully, breeders will be able to preserve the breed's integrity through selective breeding.

Kristin Rush, a breeder of Shadow Run Aussies in Phoenix and chairwoman of the breed's epilepsy program, was instrumental in bringing breeders together. Her motivation began after she produced an epileptic puppy that started having seizures at 2 ½ years of age. "When I got the phone call that 'Indy' had had a seizure, I felt like a rug was pulled out from under my feet," she says. "I didn't know epilepsy was a problem in the breed. My gut feeling was that the seizures were a toxic reaction to something else.

"I asked a lot of questions, and the more I asked, the more people became uncomfortable, even reluctant to talk to me. Finally, I started running into other breeders who had issues with epilepsy. As a support group, we found we had commonalities. We polled breeders, asking them whether they would be willing to support epilepsy education and research. Eighty percent agreed."

The mode of inheritance of epilepsy in Standard Poodles also appears to be simple autosomal recessive. Barbara Licht, Ph.D., associate professor at Florida State University, recently released research showing that in a large family of Standard Poodles epilepsy is inherited in an autosomal recessive pattern. "This means that for a poodle to inherit epilepsy, both the sire and dam must be carrying at least one copy of the defective gene," she says.

"We are continuing our work on affected bloodlines to determine if the same mode of inheritance is found in other bloodlines as well. Our preliminary



The Australian Shepherd is the first breed in which researchers at the University of Missouri will conduct a whole genome scan using SNP chips to identify the genetic mutation causing epilepsy in the breed.

work suggests that it is, although there may be additional genes that play a role as well. We also suspect that in some bloodlines, which show different kinds of seizures, there are additional 'modifier' genes that influence seizures."

Breeders are imperative in helping to advance the research, she says. "Breeders need to understand that when a dog inherits idiopathic epilepsy, researchers need information on both the sire and dam, including their prior breedings, to help track the genetic defects. For example, we need litter records for all litters related to any seizuring poodles

that they have bred. When we directly contact people who own relatives of seizuring dogs, we find that there are many more dogs with seizures that were never reported to the breeder. In particular, when the seizures are relatively mild and/or occur after the age of 3 years, the owners don't bother to contact the breeder to report the seizures."

Even in English Springer Spaniels the mode of inheritance appears to be rooted in a form of autosomal recessive, or possibly polygenic. "A partially penetrant autosomal recessive mode of inheritance would mean a single major locus with modifying genes is responsible for the mutation causing epilepsy," says Patterson. In contrast, "several genes are involved when a disease is polygenic."

The University of Minnesota research was based on a study of 45 English Springer Spaniels with idiopathic epilepsy and 74 siblings and their respective parents. The report noted that the median age at the onset of seizures was 3 years old, yet 20 percent of the dogs were between 5 and 6 years old at the time of the onset of seizures. Forty-seven percent experienced generalized, or grand mal, seizures, and 53 percent had focal-onset seizures.

"In general for most breeds, it appears epilepsy is caused by a single major recessive gene, either autosomal recessive fully or partially penetrant, or polygenic recessive," Patterson says. Breeds that fit the former category include Vizsla, English Springer Spaniel, Keeshond and Irish Wolfhound; breeds that fall into the latter are Labrador Retriever, Golden Retriever and Bernese Mountain Dog.

Patterson and his team "have found that there is likely to be an epilepsy gene in a region of dog chromosome 1 (CFA01) in Vizslas, but the results are not clear cut yet," he says. "More than one epilepsy gene or more than one type of inherited epilepsy could occur in Vizslas."

Meanwhile, the Minnesota investigators are using a whole genome scan and SNP chip analysis to study epilepsy in English Springer Spaniels. "We are also looking at genetic markers near 60 or so ion channels and receptors in English Springer Spaniels, Vizslas, Greater Swiss Mountain Dogs and Beagles," Patterson says. "Seventeen of these genes have muta-

Where to Get Epilepsy Information

For information about epilepsy and the Canine Epilepsy Research Consortium, you may visit www.canine-epilepsy.net or contact Liz Hansen, Project Coordinator at the University of Missouri, at (573) 884-3712. The Web site is maintained by the University of Missouri College of Veterinary Medicine.

tions for rare human epilepsies, and the rest are closely related genes. We are about half way through the 60 channels and have one potential positive result, but it still needs confirmation."

Though Foster has not bred a litter since the one Preston sired in 1996 because of her frustration with epilepsy in her bloodline, she realizes the efforts to collect blood samples and educate others were not for naught. "Epilepsy is so complicated and multifaceted. It's difficult because it's different for every dog," she says. "We are definitely making headway. We are getting information out, banking our dogs' DNA, and waiting a bit longer to breed. If a screening test can be developed, breeders will be able to test parents before breeding. That will be wonderful."

Rush, the Australian Shepherd breeder, agrees. "It's not the end of the world to have a dog with epilepsy. The problem exists, but with proper tools and the sharing of information, we can make better decisions."

A complex disease, indeed, epilepsy stretches the globe affecting dogs everywhere. The European Union recently awarded a grant totaling more than one million euros to researchers who will study epilepsy in more than 90 breeds. Hannes Lohi, who now works at the University of Helsinki in Finland and is part of the Canine Epilepsy Research Consortium, will receive from the University of Missouri DNA samples of extended families of 20 breeds affected by epilepsy. The potential of large-scale genetic research and investigators working together promises that epilepsy may one day become a disease of long ago. ■