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PREFACE

October 30 through November 1, 1997, were historic days in the world of dogs. They marked the largest gathering ever of scientists and dog breeders to discuss issues of canine health. Delegates from nearly every state representing all 140 breeds of dog registered by the American Kennel Club gathered with scientists from leading veterinary schools and research institutions to hear the most important findings in canine health.

Three hours were set aside during the Molecular Genetics and Canine Genetic Health Conference for a session on hereditary epilepsy. The reason for this emphasis is that the organizers of the meeting believed that the extent and impact of hereditary epilepsy on canine health and pet owner's peace of mind were greatly under appreciated.

In a survey of national Parent Clubs conducted by the AKC Canine Health Foundation in 1997, epilepsy was reported by 22 breeds as one of their top five health concerns. In an aggregate ranking of the importance of all diseases (80 diseases reported), epilepsy ranked third. In the presentation of a film on canine epilepsy at the AKC Delegates meeting in 1997, when the group was asked how many of them had experience with epilepsy, over half of the audience raised their hands. Obviously, canine epilepsy is a serious concern of Parent Clubs, breeders and individual dog owners.

Therefore, a session on this disease was one of the features of the 1997 meeting. In this white paper, the author, George J. Brewer, M.D., undertakes a brief review of hereditary epilepsy; much of which was elaborated on by the speakers at the session. We thank the speakers for the insights they provided, many of which are reflected herein. The author points out that the speakers have not reviewed this document and therefore takes full responsibility for the contents of this paper.

Overview of Canine Epilepsy, with Comparisons to Human Epilepsy

Inherited canine epilepsy is a major health problem in many breeds because of its high frequency and it potentially serious effects on pet ownership and breeder reputation. A preliminary examination of published data indicates that over 20 breeds have a serious health problem with canine epilepsy (see below for list of breeds).

There is likely to be a large amount of genetic heterogeneity in canine epilepsy. By that, it is meant that several different genes are involved. The strongest reason for believing this to be true is the demonstration, by crude mapping techniques, of seven different regions of human chromosomes that contain epilepsy genes[1] . Genetic heterogeneity is not to be confused with polygenic inheritance, in which more than one genetic defect contributes to the presence of the disease in a single patient or animal. In epilepsy, in any one patient or animal, the disease is likely to be the result of a defect in a single gene. But in the next patient or animal, the epilepsy may be the result of a defect in an entirely different gene.

One of the difficulties in studies of inherited human epilepsy is this genetic heterogeneity. Because the phenotypes of seizures and electroencephalographic abnormalities are similar from one genetic type to another, it is difficult to collect human pedigrees that are pure for one genetic form. That has made it difficult to map human epilepsy genes, one of the necessary steps preparatory to identifying and cloning a gene. The one human epilepsy gene that

has been cloned took advantage of a Finnish "isolate," a population relatively isolated from other populations, and in which the epilepsy that occurred was genetically pure[2].

Canine purebreds are "isolates" in the same sense as the Finnish population. Thus, it is expected that the cause of epilepsy in any particular breed will be due to a single genetic defect. This provides great advantages to those of us interested in finding the genes causing canine epilepsy because pedigrees collected within a breed will be pure for a single gene causation of epilepsy. This concept is no longer theory. It is borne out by VetGen's extensive experience with canine von Willbrand's disease (vWD), which is also genetically heterogeneous. We find that within a breed, vWD is due to precisely the same mutation in all dogs affected. (These data are unpublished, but I reported them at this same meeting.) Using canine pedigrees from within a single breed, the epilepsy gene in that breed can be mapped as the dog map unfolds, and the gene identified and cloned. Developing a DNA test to allow breeders to reduce the disease gene frequency then becomes a simple task. In turn, such newly identified canine epilepsy disease genes become candidates for being one of the undiscovered human epilepsy genes. In this way, purebred dogs can power not only canine gene discovery, but human gene discovery.

Background on Canine Epilepsy

Seizures, the most common neurologic disorder in dogs, can come from a wide range of etiologies[3]. An epileptic seizure has a specific neural origin, and produces excessive and/or hypersynchronous neuronal activity in the cerebral cortex. Because it is usually not possible to simultaneously record electroencephalographic changes during a seizure, historical information is usually used in both veterinary and human medicine to make a diagnosis. If the seizures are

due to a structural abnormality in the brain, they are called secondary (SES). If they are the result of a systemic insult or stress, they are called reactive (RES). If an underlying cause can not be identified the seizures are called primary (PES). PES are also referred to as idiopathic, cryptogenic, or hereditary seizures[3]. PES is the type of epilepsy we are interested in, because these seizures are, for the most part, genetically determined.

PES is thus a diagnosis of exclusion, other causes needing to be ruled out before this diagnosis is made. However, the clinical history of the dog is quite helpful. PES is the most probable when the dog is between one and five years of age at the time of the first seizure[3]. Dogs younger than one year or older than five years have a higher likelihood of their seizures being due to either RES or SES. A second useful discriminator is that the interval between the first and second seizure event (an "event" is all seizures within 24 hours) be at least one month[3]. Seizures occurring more frequently than that are more likely due to RES or SES. For a diagnosis of PES, it is required that the dogs have a normal interictal (between seizures) neurological exam. That is, the dog does not have an underlying neurological disease. Of course, a major help in establishing a genetic etiology is that the pedigree will often have at least one other first degree relative with a similar picture of seizures. In fact, this is a requirement for genetic studies of canine PES, which is at least two first degree relatives (siblings, half siblings, parents) be affected. Using the above clinical criteria for inclusion, plus an inheritance pattern, will make inclusion of dogs misdiagnosed as having PES when they really have SES or RES quite rare.

Approximately one to six percent of purebred dogs has a seizure problem[4] and most of this epilepsy seems to be genetic (PES). The incidence of epilepsy varies tremendously by breed, with a very large number of breeds, at least 20, having a high incidence of hereditary epilepsy.

A genetic basis for epilepsies in the following breeds has been established: Beagles[5]; Belgian Tervuerens[6]; German Shepherds[7]; Keeshonds[8]; Labrador Retrievers[9]; Golden Retrievers[10]; British Alsatians[7]; Collies[11]; and Welsh Springer Spaniels[12]. Several additional breeds have a high incidence of epilepsy that is no doubt genetic, but they have not been studied in depth. These include: Poodles (all three breeds), Boxers, Cocker Spaniels, Dachshunds, Irish Setters, Miniature Schnauzers, Saint Bernards, Siberian Huskies, Wire Fox Terriers[13], Berner Sennenhund and Horaks Laborhund[10]. Whenever enough data have been collected for analysis, the inheritance pattern has appeared to be most compatible with recessive inheritance.

There are many similarities between canine and human hereditary epilepsies. 1) In both species, idiopathic (hereditary) is the most common etiology. 2) The age of onset in dogs, typically between two and five years, corresponds to adult human inherited epilepsies, typically presenting in their second and third decades. 3) The seizure types, both generalized and complex partial, are common to both. 4) The response rate to therapy is similar. Thus, it can be anticipated that the genes causing human epilepsies and the genes causing canine epilepsies will be identical or strongly overlapping. Some of the same genes causing canine epilepsies are likely to be involved in mouse epilepsies. So we should see what we can learn about the genes causing human and mouse epilepsies in order to get a good preliminary overview of the genes likely to be causing canine epilepsy.

Human and Mouse Epilepsy Genes and

What We Can Learn From Them for Canine Epilepsy

Table 1 provides a list of human hereditary epilepsies, the chromosomal location of the involved gene, and the possible identity of the genes involved. Cystatin B as a cause for Univerricht-Lundborg disease is the epilepsy gene previously referred to that was cloned in the Finnish population[2]. Table 2 provides a list of single gene defects that produce epilepsy in the mouse.

Now I would like to use the information in Tables 1 and 2 to see what general lessons about epilepsy we can learn. I come up with the following points:

1. Epilepsy is generally a single gene disorder. As explained earlier, this means in an individual animal or human patient, a defect in only one gene is the cause.
2. Epilepsy is genetically heterogeneous. As explained earlier, a defect in any one of many genes can cause a seizure disorder.
3. Multiple pathogenic mechanisms exist in causing epilepsy. By this it is meant that the seizure can be initiated as a result of any one of a variety of different physiological and biochemical derangements. Some of these are:
    a. Increased excitation.
    b. Increased excitation inhibition.
    c. Neurotransmitter abnormalities.
    d. Neurotransmitter receptor abnormalities.
    e. Ion channel abnormalities.
    f. Abnormal protease inhibition.

g. Injury (from genetically caused damage).

h. Abnormal metabolism.

4. Epilepsy is a complex disease. Points 2 and 3 above make this obvious. A seizure disorder can be due to a defect in any one of a large number of genes causing disturbance in any one of a large number of systems in the brain.

Given what I have said so far, is canine epilepsy, involving so many dogs and so many breeds, with so many genes potentially involved, a hopeless morass that should be left to some future generation to tackle? Should today's breeders of dogs in the breeds affected be left to their own random fate with respect to whether some of the dogs they produce suffer from epilepsy? Should today's pet owners of dogs in these breeds be left in fear as to whether their pet will have seizures?

As the reader might suspect, I think the answer is "no" to all three questions in the above paragraph. The reasons are that molecular genetics and the structure of purebreds now give us the tools to tackle the problem.

The main reason for optimism is a point I have already emphasized: that within a breed, genetic epilepsy with a generally similar phenotype will be due to a single genetic cause. If this statement is true, it is a very potent advantage in finding the genetic cause of epilepsy within a given breed. More on that later. But is the statement true?

There are good theoretical reasons to believe the statement is true because of the strong influence of "founder effects" in purebreds. What this means is that a limited number of animals were used to start the breed, and in some cases an important stud dog was used heavily later on. The recessive disease genes carried by these "founders" then became disproportionally represented in the subsequent generations of the breed. Because any founder generally will

contribute only one disease gene to a particular type disease, for example epilepsy, the disease will be "pure," that is of one genetic type, in any breed. It is precisely this kind of founder effect in the Finnish population that led to the identification of cystatin B as a cause of human epilepsy.

However, we now have much more than theory to backup the validity of the above statement. In canine von Willebrand's (vWD) disease, we have found precisely the same causative mutation in 1,525 Doberman Pinschers that either are affected with or carry the gene for vWD. All vWD affected or carrier Scottish Terriers carry precisely the same mutations, although it is different than the Doberman mutation. All vWD affected or carrier Shetland Sheepdogs carry precisely the same mutation, although it is different than the Doberman and Scottie mutations. (I presented these data at this same meeting).

These vWD genetic results in the dog are strikingly different than the findings with human recessive diseases in a panmictic (interbreeding) population such as in the United States. There are a very large number of different mutations for cystic fibrosis, Wilson's disease, and for human vWD itself.

The data are clear: Within a breed, a common genetic disease is very likely to be pure. The lesson, extended to epilepsy, is that, within a breed, genetic epilepsy (in dogs with a generally similar phenotype) will be due to a single genetic cause.

Finding Canine Epilepsy Genes

Given the optimism I am portraying, what do we do to find the canine epilepsy genes so that DNA tests can be developed and the disease eliminated?

The first step is to collect pedigrees within a breed to be studied. The epilepsy phenotype to be studied should be defined with the help of veterinary consultants. One wishes to study only PES, that is, hereditary epilepsy. Because it is always possible that two different founders contributed different epilepsy genes, and that a breed has two different but common genetic epilepsies, the phenotype of epilepsy within the breed should be looked at. If there appears to be two different patterns, collect each separately. For example, if one type appears to have an earlier onset and be more severe, segregate those pedigrees so their genetic cause can be searched for separately.

A minimal pedigree for genetic studies should have at least two affected. With recessive disease, as most epilepsies will probably be, these affected will be siblings or half siblings. With dominant pedigrees the affected will usually be a parent and at least one affected offspring.

For the pedigree to be useful in gene hunting, DNA in the form of blood or cheek swabs must be collected from the members of the pedigree. In addition to a minimum of two affected, DNA should be collected from all parents that are available, and other affected and unaffected siblings. Other branches of the same pedigree, if they contain more affected, may be very useful. Approximately ten to twenty pedigrees should be collected, the number depending on the quality of the pedigrees.

The second part in all this is to find a molecular geneticist or molecular genetics team to do the gene search. Part of the barrier to this step is funding. It takes $50,000 - $65,000 per year

to put a good, full-time person to work on a project of this type, and it may take some time (multiple years) to find the gene. Thus, sources of funds, such as the breed club, individuals, the AKC Canine Health Foundation, the Morris Animal Foundation, etc. need to be found for each project.

The type of molecular genetic work that will be done includes looking at candidate genes for the cause in a particular breed (for example the known mouse genes - Table 2 - are all candidates). If this fails, the canine gene can be mapped on the dog genome map as it emerges in the next two to three years, and then by comparing the position on the human map, new ideas generated on the likely genetic cause.

In the epilepsy session at the meeting we heard from two experts on canine epilepsy, Dr. Podell and Dr. Parent, who helped us understand the problems of diagnosis and understand how inherited and non-inherited epilepsies can be separated. They also told us what is known about the sub-types of canine epilepsies that may guide us in beginning to identify different genetic types. From Dr. Licht we heard about the disease from a breeder's perspective, and what one group of determined people, and a committed breed club (The Poodle Club of America), are doing about the problem in their breed. They are tooling up to do a very rigorous and thorough study of epilepsy in the Poodle.

Summary

We believe the session on epilepsy served the main purpose for which it was intended – to call attention to this important canine health problem. With the new information that is rapidly developing about mouse and human epilepsies, it should be possible to begin identifying some of the canine epilepsy genes in the near future. Once the genetic cause is identified it is straightforward to develop a DNA test that will detect the mutation in gene carriers as well as in affected canines. We can anticipate that generally hereditary epilepsy within a breed will be pure, that is due to a single genetic cause. However, across breeds there will likely be five to ten different epilepsy genes, all of which have to be searched down in order to offer DNA tests for each one.

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