

DEGENERATIVE MYELOPATHY in Chesapeake Bay Retrievers

The ACC Trust sponsored Dr. Sam Long's presentation on Degenerative Myelopathy in Chesapeake Bay Retrievers at the 2008 National Specialty in Massachusetts on October 9th. Dr. Long presented his research to members of the ACC before his findings had been published and before their presentation to the medical community in November. The following is a synopsis of his seminar

"Degenerative Myelopathy in Chesapeake Bay Retrievers, Canine Lou Gehrig's Disease?" By Sam Long, BVSc PhD DipECVN, Section of Neurology and Neurosurgery, University of Pennsylvania School of Veterinary Medicine, 2008. Prepared by the ACC Health Committee.

Degenerative Myelopathy was first described in German Shepherds in the US by Averill et al, 1973 and in the UK by Duncan and Griffiths, 1975. It has since been found sporadically in other large breeds as well as in Corgis and Boxers. Studies describe DM as a T3-L3 myelopathy; clinically showing in the hind limbs with scuffed nails, decreased ability to coordinate muscle movement, and decreased sense of position and movement. DM is a disease of the spinal cord which is comprised of White Matter, Axons (wires from the brain to the muscles) and Myelin (insulation around the wires). In this disease the Myelin degenerates and dorsal nerves degenerate. Additionally the Vestibular and Red nuclei in the brain stem are affected. This is found post mortem by histological evaluation. The age of onset documented was 5-11 years and progression was found to be slow. Initial diagnosis rules out other causes such as intervertebral disc disease, tumors of the spinal cord and infections within the spinal cord. Tests utilized for diagnosis include MRI, myelography with or without CT, and post-mortem histology examination. The cause has been unknown, treatments ineffective, and prognosis hopeless.

The first case seen at Penn in March of 2006 was a 9yr old male Chesapeake Bay retriever, DC Distagon, who had been used extensively for breeding. When two litter mates were also affected as well as others, including prominent dogs used in breeding, the question arose "Is DM a problem in CBRs?" The Chesapeake community responded by proposing and funding a project which aimed to 1) describe the disease in CBRs, 2) estimate how common the disease is in CBRs, 3) identify mode of inheritance, 4) collect blood for sequencing, 5) perform genome-wide association studies to identify gene(s) of interest, and 6) develop a before death diagnostic test.

MATERIALS AND METHODS: Dogs were evaluated looking at their history, signs and neurological examination. Post-mortem examinations were done to establish definitive diagnosis. A data base was created to track dogs and generate pedigrees. The pedigree collection/analysis was to evaluate prevalence and mode of inheritance. Blood samples were obtained (ACC Field Trial pictures were included) from field trials and postings on the ACC website. The final step was to present these samples to the Univ. of Missouri for inclusion in their study.

Dr. Long began with the **Clinical Information and the Strength of Diagnosis** – a collection of history, signalments and neurological examination of the dogs -. The "Strength of Diagnosis" provided difficul-

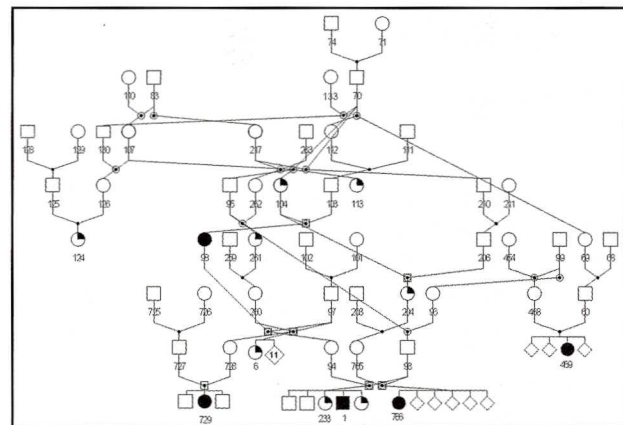
ties for the criteria of inclusion because many times full diagnostic tests were not performed, with multiple conditions presenting similarly to DM. Therefore for sequencing purposes it was essential to only include cases definitely affected. The research team determined a Stringency of Diagnosis comprised of 4 levels. The highest level - Level 1- consisted of histopathologic confirmation. Level 2 included clinical signs and MRIs with no compressive lesion detectable. Level 3 included clinical signs and myelographs with no compressive lesion detectable. And finally, Level 4 represented clinical signs alone, including progressive upper motor neuron paresis (muscle weakness) and ataxia (problems with muscular coordination).

The Pedigree Analysis and Prevalence portion of the study tracked dogs and evaluated pedigrees to evaluate the prevalence and mode of inheritance. Researchers studied 32 affected cases. But of those, 14 dogs had only "consistent history but no definitive investigation" leaving only 18 dogs "with diagnosis"(five Level 1, seven Level 2, and six Level 3 dogs). Using all 32 dogs, which were born between 1994 and 2000, compared to the total number of CBRs registered with the AKC the incidence of CBRs affected with DM was 9 per 10,000 (0.09). But looking at the data year by year, there appears to be a higher and possible increase in number since it is possible that dogs born in 2000 are just too young to be showing symptoms yet.

Chart #1

Year of Birth	No of Affected Dogs Diagnosed with DM	Total CBRs Registered with AKC	Incidence
1994	1	5198	0.02
1995	6	5069	0.12
1996	3	5540	0.05
1997	6	5204	0.12
1998	7	4685	0.15
1999	7	4594	0.15
2000	3	4665	0.04
Total	32	34955	0.09

Dr. Long's final step was the **Collection of Blood Samples**. Researchers obtained 138 samples through draws at the Field Specialty as well as appeals on the CBR website. Evaluations of these samples found 27 affected dogs and 111 unaffected related and unaffected non-related dogs. By requesting 5 generation pedigrees from the submitted dogs, researchers were able to enter 1011 dogs into the database. For the first pedigree analysis of the affected dogs (Stringency Level 1 -4), the research team included 796 dogs going back 14 generations looking for a common ancestor. That graph provided no conclusions so 9 smaller family trees were created from 3 generations of affected dogs. The following family tree pedigree analysis below shows that 1) the percentage of affected dogs within litters is 25%, 2) that not every dog affected has an affected parent, and 3) that both males and females are affected. This preliminary analysis is suggestive of autosomal recessive – with or without complete penetrance.



Blood samples were then submitted to the ongoing University of Missouri project led by Dr. Joan Coates in collaboration with Broad Institute of MIT and Harvard. This project was a **Genome Wide Sequencing Study** with fine mapping of multiple breeds. The basic principles of sequencing involve recombination during meiosis. Chromosomes break apart and swap during cell division during the formation of eggs and sperm. Linkage analysis relies on the tendency for genes to be inherited together in the same region in recombination. Genetic markers trace recombination based on other genes, restriction of fragment length, Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs).

The first Stage is to genetically compare the Affected and Unaffected dogs of one breed. After an area is identified then Stage 2 is Fine Mapping across dog breeds attempting to find a small region with just a few genes. The U of MO study used the Pembroke Welsh Corgi (38 Affected and 17 Controls) for Stage 1 and then for Stage 2 of Fine Mapping added Boxers (13 A & 42 C), GSDs (14 A & 55 C), CBRs (19 A & 18 C), and Rhodesian Ridgebacks (12 A & 10 C). Initially raw mapping of haplotype and SNP using the Corgis showed a spike GGGC but when fine mapping of multiple breeds was added 0% of affected Rhodesian Ridgebacks had this. Researchers returned to the data and found that Concordant Haplotype GTTAC showed 100% association and there was a Superoxide Dismutase1 (SOD1) mutation at A with 100% association in affected dogs. (See chart #2 & 3) This data suggested that SOD1 was the candidate gene. The SOD1 gene codes for a protein and mutations in it are known to cause Amyotrophic Lateral Sclerosis (ALS/ Lou Gehrig's disease) in humans. This mutation was identified in all breeds with DM. When the chromatograms are aligned in the SOD1 exon 2 segments a G--to--A transition is contained which predicts a glutamate-to-lysine missense mutation. Therefore this mutation results in a change in the amino acid sequence of the SOD1 protein. The SOD1 protein is a free radical scavenger; its job is to neutralize superoxide radicals, which can damage cells if their levels are not controlled. SOD1 is found abundantly in the spinal cord and mutations in it have been linked to neuronal damage. Over 120 different SOD1 missense mutations have been identified in ALS patients. 20% of Familial ALS (which accounts for 5-10% of all ALS) and approximately 5 % of Sporadic ALS is linked to SOD1. Until now, no spontaneous animal disease models had been found. The results of this study found the mapping of the 5 dog breeds shows the best association at the SOD1 mutation also.

For the genotypes of CBRs all 138 blood samples were submitted and 105 were sequenced. Of those 105, all 25 affected dogs tested A/A. Of the remaining 80 unaffected dogs, 17 were A/A (too young to show clinical signs), 44 were A/G and 19 were G/G.

Chart #2

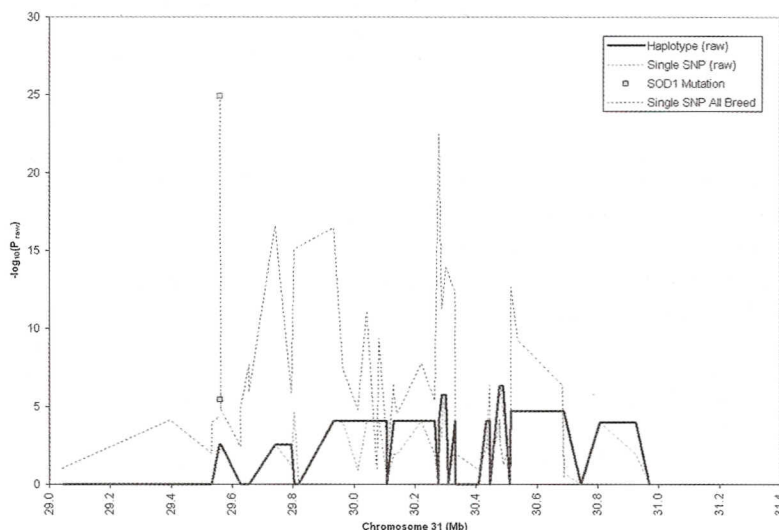


Chart #3

CASES	SOD-1 haplotype (%)			SOD-1 mut. (%)		Most associated haplotype (%)		
	GTTAC	ACCGT	Other	A	G	GGGC	TAAA	Other
Pembroke Welsh Corgi	100	0	0	100	0	100	0	0
Boxer	100	0	0	100	0	100	0	0
Rhodesian Ridgeback	100	0	0	100	0	0	100	0
Chesapeake Bay Retriever	100	0	0	100	0	100	0	0
German Shepherd	100	0	0	100	0	75	13	12
CONTROLS								
Pembroke Welsh Corgi	90	10	0	76	24	68	32	0
Boxer	100	0	0	63	37	87	13	0
Rhodesian Ridgeback	95	0	5	—	—	35	40	25
Chesapeake Bay Retriever	72	19	9	43	57	86	14	0
German Shepherd	98	2	0	33	67	44	56	0

Conclusions: From this study we can conclude that DM in dogs appears to be the result of a missense mutation affecting exon 2 of the SOD1 gene which leads to a dysfunctional SOD1 protein. This SOD1 protein accumulation in the spinal cord cells can be detected in dogs similarly to that in human ALS patients. Though originally defined as affecting the hind limbs, DM, if left to progress, will ultimately include clinical signs in the fore limbs causing tetraparesis/plegia (weakness to paralysis in all four limbs). The mode of inheritance in CBRs fits best with the autosomal recessive model which may be incompletely penetrant. And from this work, a diagnostic test is now available through Univ. of MO and OFA that is being marketed as identifying a major risk factor.

What's next? These results and the conclusions that have been drawn help pinpoint further work that must be done. More study of the region is needed to determine if other mutations exist. It is also important to explore other associated regions in the genome to determine the impact on degree of penetrance and for the age of onset. Continued examination of the central and peripheral nervous system components of DM is also necessary. Finally researchers must continue to investigate other possible associated phenotypic markers for the disease and research possible therapeutic interventions.

Dr. Long ended his presentation thanking foremost the dogs, then their owners and breeders, the ACC, the Trust and the Health Committee. Next, he took questions from the audience. Someone asked "Could DM be an autoimmune disorder?" Dr. Long stated that the histological findings do not support that theory. Another asked about the possibility of other genes being involved in affected dogs "Have we found the right gene?" Dr. Long replied that personally he is pretty much convinced they have identified THE gene, SOD1. He was also asked questions

ACC's presentation of the American Kennel Club's 2008 Outstanding Sportsmanship Award

about breeding. He first stated "Don't exclude affected dogs from the breeding pool completely. You have a small breeding pool; we must avoid genetic bottlenecks." He then emphasized that we need to test as many dogs as possible, preferably at a young age. And finally, we can reduce the frequency of the A allele by breeding affected and carriers to normal dogs. He was also asked "Would DM progress to affect swallowing and breathing?" He stated that in a Corgi (a much smaller dog that can be kept alive longer since it is much easier to support) it was occurring before the dog was euthanized. He also discussed the importance of the A/A dogs over 10yrs who are not showing any symptoms. These dogs are vital to the continuing research (their blood work is free at the Univ. of MO). In this lecture, he showed many heart wrenching videos and slides of affected dogs, showing what to look for and how the disease affected them. Hopefully with continued research we can find answers and treatments.

The Health Committee is recommending the testing of Chessies- for info go to www.offa.org/dnatesting/. The breeding guidelines listed there state "breeders should take these results into consideration; however they should not overemphasize the test results". At this point "the mutation (A/A) can only be interpreted as being at risk of developing DM within the animal's life". The Univ. of MO site www.caninegeneticdiseases.net/DM/mainDM.htm also has a link for sample submission. They also discuss the "A" allele and recommend to "consider dogs with A/A or A/G to have a fault, just as poor top-line or imperfect gait would be considered faults". They also recommend that "the test result is one factor among many in a balanced breeding program". They stress in bold that "**an overly aggressive breeding program to eliminate the A/A or A/G dogs might be devastating to the breed as a whole**". The Health Committee will continue to report on the results of the continued research. If you have questions after reading this synopsis, you may mail or email (curlygrl@ptd.net) them to the Health Committee for consideration in further articles.

The American Chesapeake Club announces the 2008 recipient of the American Kennel Club's Outstanding Sportsmanship Medallion to Susan Cone. The award was presented at the National Specialty Show of the American Chesapeake Club in Fitchburg, MA on Oct. 9.

Sue Cone has long been an active member of the ACC. She was instrumental in heading a committee to work with researchers to develop a test for PRA. Sue has served the ACC on the Show Committee and has worked as Obedience Chair for our National Specialties on numerous occasions.

Sue's personal accomplishments in the area of AKC obedience are unparalleled within the club. Sue has titled many dogs and has achieved multiple OTCH's. She has helped other exhibitors to also be successful in obedience contests.

Recently, and importantly, Sue has become a one-woman liaison to several organizations fighting anti-dog legislation. The club members receive daily information on upcoming legislation in their area and who to contact with their comments. This provides a valuable service to the Club.

The American Chesapeake Club is honored to have Sue Cone as a member.

From Sue:

It's been an honor and a joy to work for our wonderful breed, and the ACC over the decades. I've learned so much, made so many wonderful friends, and known so many outstanding dogs! Thank you all for giving me this award. I cherish it.

Sue

ACC Charitable Trust

ANNOUNCEMENT

ACC Health Committee

We have just received word that our good friend Dr. Sam Long, PhD, will be leaving University of Pennsylvania in January, to take a position at University of Melbourne in Australia. We are grateful to Dr. Long for his interest in our Chesapeakes and the time and effort he put into the DM study, and wish him all the best in Melbourne!

Dr. Long plans to try to keep coordinating with ACCCT/ACCHC on the continuing DM research. However, research will be conducted at University of Missouri. Any samples for ongoing research (CBR's with a presumptive diagnosis of DM made by a veterinarian, or CBR's age 10 or older) must be sent directly to University of Missouri, for no-charge DNA testing. Info. and submission forms at: www.caninegeneticdiseases.net/DM/sampleDM.htm. If you have questions contact ACC Health Comm. member Briedi Gillespie at bgillespie@rossvet.edu.kn or Lois Wida at curlygrl@ptd.net. REMEMBER - continued testing of diagnosed or older dogs is VERY important to this on-going research!

If you submitted samples during the initial research period (at the 2007 FT Specialty or mailed in), all those samples should now be processed. To get your free test results go to www.caninegeneticdiseases.net/DM/sampleDM.htm, use link at bottom of page, and specify "U of Mo" in the form you submit via email.

Regular DM screening test kits will continue to be offered at www.offa.org at the online store.

Diane Mazy / ACCCT