State of the German Shepherd DM

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GSDM Genetics:

Our project on the genetic aspects of GSDM has led to the development of the DM Flash test which is based upon RAPD PCR technology. Using this, we have found that the DM Flash test is a great diagnostic test since it has an overall sensitivity of 96% and specificity of 99%. In a clinically affected dog, a positive test indicates a high probability that the dog has GSDM. Less than 1% of positive dogs will not have GSDM as part of their disease. As such, we now recommend GSDM testing in GSDs that are the right age and who have classic clinical signs. If they respond to medication, then we do not need to work them up further. If they do not respond, then we recommend that a workup be performed to rule/out other diseases. Of course, this is based upon the concept that the disease is not acute in nature which would not be compatible with GSDM.

On the other hand, if a clinically affected dog is negative, there is still a 4% chance that the dog still has GSDM. These dogs should have a workup to rule/out other diseases and confirm the presence of GSDM using those criteria we have previously explained. These include besides the clinical signs, elevated lumbar CSF protein, negative EMG except for altered spinal evoked potential, and negative imaging (preferably spinal MRI). If GSDM is confirmed with these tests, we treat as such. However, is another disease is found, we treat that as the likely cause of the disease.

The DM Flash test (nor any other current test) is not a good screening test for the general population since a screening test should fit 3 criteria: 1) the test should have a high predictive value of a positive test (in the general population this is only 13% since most positive GSDs will never get GSDM); 2) the test should be relatively inexpensive (here we are okay); and 3) the knowledge of the result of a positive test should benefit the patient (which it does not, since there is nothing to prevent the later development of the disease). So, we do not recommend using the current genetic tests including the DM Flash test as a screening test for normal dogs. Our studies have shown that around 20% carry the GSDM trait we track in the DM Flash test. Since we think our test only finds the homozygous condition (based upon sequence data in our early investigations), it supports that the trait we track is recessive and a common mutation. However, RAPD analyses do not point to a specific gene location so we do not know for sure from which gene, the change comes from. Even so, this is a consistent change within the gene associated with animals who are at risk of developing the disease. We can explain 91% of the reason dogs will develop GSDM based upon a positive DM Flash test.

Recent emerging data indicates that utilizing genomic wide screening techniques like SNIP arrays or RAPD analysis, may find common mutations in disease; however, these may be “synthetic associations” where these patients are at risk, but the development of disease is rare even in this group. This is the case in GSDM. While 20% (effectively 25%) carry the common trait in the association, only 2% actually get the disease (10% of the risk population). As such, the rare genetic trait which puts GSDs at high risk for GSDM is being hidden by the common trait. We do not yet know what that hidden rare genetic mutation is which really leads to GSDM, only the common trait that point toward it. For clinically affected dogs, this does not have much consequence; but for normal dogs, finding this rare mutation is what is needed to develop an effective screening test. Hopefully, by collaborating with other researchers, we will eventually narrow the prospects to find this rare mutation.

We have documented several genetic changes which appear to be related to GSDM, but are not necessarily part of the DM Flash test. Our initial investigates demonstrated at least 3 potential changes in the DRB1 regions of the MCHII genes. One was the initial priming error we uncovered using DRB1 primers which formed the basis of the initial testing we performed. In addition, this led to a photometric change which was a second phase test. Sequencing the products of DRB1 showed a homozygous point

homology in the second hypervariable region of the gene. While this was never the focus of the DM Flash test, this is one change that we consistently found in GSDM dogs. This is in the region of DRB1 which tends to regulate overall immunoreactivty in patients and is postulated to lead to an overall increase in immune sensitivity which appears to be part of the GSDM condition. We also found a mutation in another which leads to a deletion error in the region that encodes folding of myelin basic protein (MBP) and may be responsible for increased immunoreactivity to MBP seen in the disease. Additional gene changes we noted in the APOE and IL4R genes which is consistent with the hypothesis that GSDM may be similar to Primary Progressive Multiple Sclerosis (PPMS). Initial examination of SOD1 did not show any changes consistent in GSDM. However, in light of the data from the Broad Institute and the University of Missouri, we did look at the encoding region of SOD1 (a region we had not previously covered) and found that at least 60% of the patients we diagnose with GSDM have a change in that region. Again, this SOD1 change does not appear to be as specific in GSDM as the DM Flash test and is probably a common mutation, not the rare mutation that would have high-risk significance.

So, while we know much more about various mutations that occur with some frequency in GSDM, we may not yet know for sure which of these changes is the most important nor even if any are what really determines the ultimate risk of GSDM. That is not uncommon when dealing with complex genetic diseases. On the other hand, we do have a functional diagnostic assay (the DM Flash test) which fulfills the requirements to help diagnose the disease.

Treatment Options:

Treatment of GSDM still remains elusive. The use of antioxidants (including acetylcysteine) and exercise continue to provide the best options for improvement and progression prevention. However, once the patient has lost the ability to stand, these measures are not enough to reverse the disease process. We still feel that emerging stem cell technologies will be the best hope to repair damage and prevent further deterioration; however, current methodology are lacking. Canine adult stem cells which are histocompatible with the host do not become functional cells. They only provide temporary relief of the signs due to growth factors which they produce and release. On the other hand, since they do have influence on glial scarring, it is hopeful that they can be coaxed into a longer stand benefit through genetic modification or growing them under the right conditions. Endogenous stem cells may also be induced to help with healing. Emerging data suggests that neural stem cells may be under cyclic nucleotide control. Compounds which elevate c-GMP tend to promote stem cell replication and may be a key to helping improve patients. We are about to begin a trial using Viagra which has been useful in treating ischemic stroke patients by inducing endogenous stem cells to proliferate and help repair the nervous system.

Overall Conclusions:

We continue to make slow progress on the disease; however, GSDM is complex. While some people have begun to suggest that DM in all breeds is related. We feel that GSDM is still a specific disease which affects primarily the GSD and related breeds. The disease in some un-related breeds (such as Corgis and Boxers) appears to have a different basis from our work. They appear different in RAPD analysis and the DM Flash test must be modified to identify these patients. Even though some GSDs have a change in SOD1, not all of the GSDM patients we diagnose with the disease based upon all our clinical criteria have an SOD1 change. Moreover, the pathology of the GSD in GSDM does not support the motor neuron hypothesis. This is the same group that originally suggested GSDM was a nutritional disease. Now, they believe it is genetic. So, that is progress. You cannot look at a single common mutation and understand the disease. You also cannot ignore the rest of the data about GSDM and its clinical picture. Amyotrophic Lateral Sclerosis (ALS) has some association with SOD1, but that is not the sole problem even in ALS. ALS patients do not have elevated protein in the CSF and they have

characteristic changes in motor units either in the spinal cord or brain which are indicative of the diseases. Neither of these changes has been seen in the GSD. So, while ALS does not have an immune basis, PPMS and GSDM do. As such, there has been no compelling evidence to change our position about the basic nature of GSDM as a disease similar to PPMS. This may not be true in the Corgis or Boxer, but it just suggests that neurodegenerative diseases can come in many forms as it does in people.

We remain committed to finding new treatments and also in searching for the high-risk genes in GSDM so that we might develop a screening test for the general normal dog population. However, this may require collaboration with other investigators. My research lab exists, but only barely since space was shifted. We are currently able to process the DM Flash tests, but only because the numbers have remained low. Mostly, it is I who personally performs them. Even so, I think the future is bright and we are actively seeking funds to continue the research and expand the program to new areas.

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Respectfully submitted,

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