WHAT IS LD AND WHY DO WE TEST OUR DOGS FOR THIS DISEASE?

Explaining the New Mutation-Based Test for Exfoliative Cutaneous Lupus Erythematosus (ECLE aka Lupoid Dermatosis)

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Introduction

Breeders of German Shorthair Pointers (GSP) have known that exfoliative cutaneous lupus erythematosus (ECLE) is an awful, incurable skin disease for a long time now (Figure 1). We know that it is the same as cutaneous lupus erythematosus (CLE) in humans. Ten percent of human patients with CLE develop systemic lupus, i.e. kidney disease as a result. Sadly, every single GSP with ECLE will develop kidney disease associated with LD. To date, we have not seen mild forms of this disease nor have we seen any GSPs with LD that did not develop kidney disease. It is clear that we must eliminate the disease from the breed and at the same time what we learn in dogs may help find a cure not just for the dogs but also for humans with the same devastating disease.

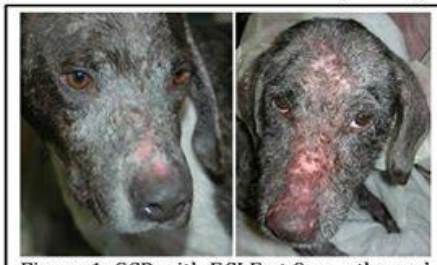

Figure 1. GSP with ECLE at 9 months and 2 years of age. Note the progression of disease with no improvement despite treatment attempts.

For years, we have been searching for the culprit gene until it was recently (Nov. 2019; paper submitted for publication) discovered by our collaborator's lab using samples from affected dogs that had been submitted to our lab. Until now, we had been offering a marker test, which can never be as accurate as a mutation-based test. However, it was helpful, and even though there were some incorrect calls, the number of affected dogs has decreased because so many breeders opted for marker testing. The most common missed call was that dogs were considered carriers with the marker test and then showed up as normal (clear) with the mutation-based test. How does this happen? Marker tests and mutation-based tests are not the same.

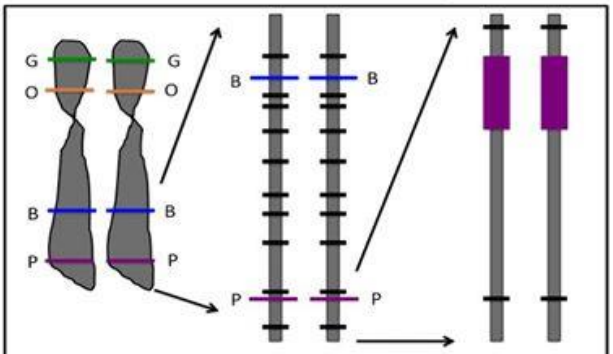

Figure 2. Left: A pair of chromosomes is shown with four fictional genes: green, orange, blue, and purple. You inherit one chromosome from your father and one from your mother. Center: A region on the chromosomes is magnified to show markers (black bars) that lie between the genes (B/P). Right: A specific region around the "purple" gene is magnified even more to demonstrate how one marker (top) can lie closer to the gene than another marker (bottom).

Marker vs Mutation

While genes code for traits like coat color, hair quality, or eye color, or specific enzymes for example, markers are located within genomic regions that generally don't code for anything (non-coding regions) but are inherited just like genes. What makes these markers useful is that they are plentiful and evenly distributed between genes and can be variable in different dogs (Figures 2 and 3). During the process of identifying a disease-causing gene we are making use of these markers that are all over the genome and hope to locate one marker nearby the actual disease gene. Again, this marker does not cause the disease but it is what we call "linked" to the disease-causing gene. For example, in the

cartoon in Figure 3 the purple bar is the gene with the actual disease-causing mutation on one chromosome but not on the other one (yellow arrow pointing to the green bead that should be yellow like the one on the other chromosome). Figure 3 also shows how these markers are located in the non-coding region between the genes. These markers are helpful to us as they often are variable between dogs (represented in the cartoon by a difference in the number of the beads). For example, there are 12 beads on the chromosome with the mutation and only 6 beads on the normal chromosome. Additionally, note, that the one marker (red/green) is closer to the purple gene than the other (yellow/blue) marker. Why does this matter? Because there is a phenomenon called "crossing over". Chromosome pairs can break at the exact same spot and reattach on the opposite strand. In Figure 3, imagine there was a break between the purple gene and the more distant marker and there was the same break on the other chromosome. After reattachment, the number of beads on the chromosome with the mutation has changed and is now only 6 beads. The chances that such a break occurs is higher the further a marker is from the actual disease-causing gene. This explains why marker tests can be wrong. Remember labs that run marker tests don't yet know where the actual gene is; they just know it is in the vicinity of the marker that is tested for. The farther this marker is from the actual disease-causing gene the higher the chance that this marker gets detached from the mutation carrying chromosome and therefore the higher the percentage of false results.

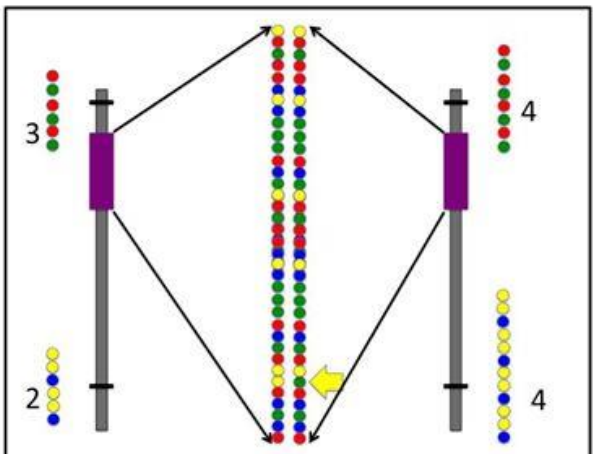

Figure 3. Further magnification of the region depicted in Figure 2, right side. Four different "nucleotides or bases" make up the code in the DNA of every living being. Here they are shown as red, blue, green, and yellow beads. Even the markers are coded using the same system.

GWAS

Originally when we started to look for the gene that causes ECLE, we performed a genome wide association study (GWAS). This is a method by which DNA from affected dogs is compared to that from normal dogs of the same breed, in this case, GSPs. This cartoon analogy might help in explaining a GWAS: Say you like a donut made by a company called DoNAut and you would like to visit the factory but none of the boxes or their trucks are labeled with the origin of the donuts. So, you take all your friends and post them with a clip- board in hand at all of the highway exits in the US. Every time they see a DoNAut truck go by, the make a note of it on their clipboard. After a week, you call them all back and see that the person in Tallahassee, FL saw 1 truck in one week, as did the person in San Diego, CA and Houston, TX. The people stationed in Philadelphia, PA and San Francisco, CA saw 5 per week, and the people in Minneapolis, MN, Kansas City, KS, and Nashville, TN saw 50 trucks per week. The person in Chicago, IL saw 400 and the person at the Milwaukee, WI exit saw 900 in one week. At this point, you know the DoNAut factory must be near Milwaukee. You have found a region but not the factory (gene) yet. The GWAS allowed us to locate a region of DNA that looked promising for harboring the affected gene. Just to give you a perspective of the complexity, the DNA from 15 affected and 21 normal dogs was put on microscopically small chips that contained 127,000 pieces of DNA. These pieces of DNA on the chip correspond to known sites (markers) on the dog's DNA. Our GWAS generated over 4.5 million bits of data

that computers and our analysis had to comb through to find major differences in the DNA from affected versus normal dogs!

Why are you now receiving results that may differ from the original results?

It is important to remember that testing, even when using a marker test, can greatly assist in reducing the number of affected animals born without getting rid of carriers that might carry a great set of other trait genes. We are now delighted to offer the new and improved mutation test to further assist with planning of your breeding program. Please do not hesitate to reach out with questions.

The original tests were both marker tests. The first one was published (you can view it here: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3230530/pdf/nihms-339857.pdf). Shortly after publication, we realized it was not accurate enough because the variation was too far away from the gene resulting in frequent breaks (i.e. crossing over). Therefore, we continued to search. We found a second marker (closer) but never published this because we knew it was not the mutation. We were also aware that there were some incorrect results due to crossing over. However, the search for the actual mutation continued and fortunately, with some help from our collaborators, we were able to find what we believe is the mutation. The original results that you received before November 2019 were based on the marker tests. Retesting of these dogs was performed using the new mutation-based test. Overall, the majority of misidentifications were carrier dogs that turned normal (almost 20%). There were 3 dogs that tested as carriers originally but then turned out to be affected with the new test. However, we had already known that because of their biopsy reports. There were 4 normal dogs that became carriers and two that tested as affected became carriers which was known to us as these two dogs are owned by our colleagues. A publication describing the gene and the mutation/variant has been submitted and as soon as it has been published, we will have the link posted here.

We appreciate the complexity of this specific condition and testing. We are grateful for the support of the GSP community and dedicated breeders who, like us, are doing the best we can with the information available. Fortunately, technology has advanced since the original marker test, and this should further help with understanding the genetics of this condition. The kidney disease component still needs to be further elucidated, and we continue to work on understanding and preventing ECLE in dogs.