

## Dermatomyositis (DMS) Learning Exercise Answers

### Answer to Exercise #1:

True! You could breed her to any stud and all pups would be low risk. Even if you used a dog having the AABB genotype (so far 100% of dogs with this genotype have been affected) all pups would be AaBb, which is low risk!

### Answer to Exercise # 2: – A and D are TRUE.

- a. TRUE. *DLA-DRB1\*002:01* is a risk allele. See “d” below. The normal (aka “wild type”) alleles of loci A and B are represented by lower case letters, a and b, while the risk alleles are A and B.
- b. The 002:01/023:01 AaBb genotype is low risk, not moderate. See <http://americanshetlandsheepdogassociation.org/dermatomyositis/> for a listing of risk assessments for each possible genotype. Besides, there is NO genotype that should prohibit a dog from being bred – just have to find a mate of the correct genotype to produce low risk pups. (See either the DMS genotype calculator or go to the Punnett square link at the above link.)

Remember, the risk interpretation applies to the risk of an individual dog being tested to develop clinical signs of DMS, and does not indicate the risk of that dog producing affected puppies. It is important for breeders to know and share each dog’s genotype, so progress can be made in decreasing the incidence of DMS.

- c. This bitch does have a low risk genotype; however, to produce a litter with all low risk puppies she should be bred to a stud with aabb genotype. Breeding her to any stud with “A” or “B” alleles, even if that dog has a low risk genotype, would mean that each pup has a chance of inheriting a moderate to high risk genotype. Each breeder must take multiple factors into consideration for breeding and may have to accept various amounts of risk. Go to the above weblink and click on the Punnett square link or use the DMS Genotype calculator to see the various possibilities of genotypes.
- d. TRUE. Although the *DLA-DRB1\*002:01* allele is the most common in Shelties (78% of those studied [1] had at least one copy and 60% were homozygous for it), there are at least 2 other alternate alleles, with 023:01 and 015:01 being the most common alternates. If all things are equal, and you have a choice between a stud with 002:01/002:01 and another with 002:01/023:01 or 002:01/015:01 either of the latter would be more desirable. Over a period of years, breeders could work toward increasing the number of dogs with 023:01 and 015:01 alleles (and any other alternates that might be found as more dogs are tested).

To put this in perspective, Collie breeders have few if any other options for the DLA as over 90% of Collies tested had *DLA-DRB1\*002:01/002:01*.

It is known that *DLA-DRB1\*002:01/002:01* (homozygous for 002:01) is associated with increased risk and that heterozygosity (2 different numbers like *DLA-DRB1\*002:01/023:01*) confers lower risk. **Does homozygosity of alleles other than 002:01 cause increased risk?** As of May, 2017, there were so few dogs with homozygosity of alternate alleles (ex. 023:01/023:01 or 015:01/015:01) that the answer is unknown and since there are so few other alleles in the Sheltie population, it may be years before that question can be answered.

**Answer to Exercise # 3:**

Below is a page from the Punnett squares posted on the ASSA website at:

<http://americanshetlandsheepdogassociation.org/dermatomyositis-dna-test/> (click on the Aabb genotype to bring up the Punnett squares).

		<u>Aabb</u>			
		<u>Ab</u>	<u>Ab</u>	<u>ab</u>	<u>ab</u>
<u>AaBb</u>	<u>AB</u>	<u>AABb</u>	<u>AABb</u>	<u>AaBb</u>	<u>AaBb</u>
	<u>Ab</u>	<u>AAbb</u>	<u>AAbb</u>	<u>Aabb</u>	<u>Aabb</u>
	<u>aB</u>	<u>AaBb</u>	<u>AaBb</u>	<u>aaBb</u>	<u>aaBb</u>
	<u>ab</u>	<u>Aabb</u>	<u>Aabb</u>	<u>aabb</u>	<u>aabb</u>

2 AABb: 4 AaBb: 2 AAbb: 4 Aabb: 2 aaBb: 2 aabb

12 low risk: 4 AaBb, 4 Aabb, 2 aaBb, 2 aabb with homozygous or heterozygous *DLA-DRB1\*002:01*

2-4 moderate risk: 2 AAbb with homozygous or heterozygous *DLA-DRB1\*002:01*  
0-2 AABb with heterozygous *DLA-DRB1\*002:01*

0-2 high risk: AABb with homozygous *DLA-DRB1\*002:01*

Each pup would have the risk of inheriting one of the above genotypes. 12 of the possibilities are all low risk regardless of the DLA being homozygous or heterozygous. Unfortunately, each pup also runs a slight risk of inheriting moderate or high-risk genotypes.

Remember, the risk interpretation applies to the risk of an individual dog being tested to develop clinical signs of DMS, and does not indicate the risk of that dog producing affected puppies.

**Answer to Exercise #4:** C and D are TRUE.

- a) FALSE. DMS is one of those diseases that cannot be caused by one parent. The affected pups must have inherited risk alleles from each parent. Take the genotype of 002:01/002:01 AaBB. This is a high-risk genotype - in the Evans, et.al. paper, 90% of dogs with this genotype had DMS [1]. What do we know about the genotypes of the parents? Since the DLA is homozygous, we know that each parent had at least one 002:01 allele, and since the dog had BB, each parent had at least one of those. We do not know which parent contributed the "A" or the "a". Each parent could have had the low-risk genotype, 002:01/002:01 AaBb.
- b) The DMS status of each pup doesn't necessarily mean that all should be sterilized. Make that decision as you normally would. There is NO genotype that cannot be used for breeding to produce pups with low risk genotypes! If you have a dog with a high-risk genotype that otherwise has desirable qualities, you could breed the dog to one with aabb genotype and all pups would have low risk genotypes. With time (years), breeders can work toward decreasing the number of dogs with risk alleles while maintaining genetic diversity and desirable phenotypic traits.
- c) TRUE. See above.
- d) TRUE. See above.

**Answers to Exercise # 5**

- a. 18, one of which developed DMS. (In Collies, 44 had this genotype and 1 of those developed DMS. This is a low risk genotype. Remember, low risk does not mean no risk, but it is the best we have. The two affected dogs with this genotype could also have been misdiagnosed, as there are several skin conditions with similar presentation.
- b. aaBbCC – 32 Shelties in this group. aaBbCC = 002:01/002:01 aaBb
- c. 100%
- d. This genotype was so rare (1 normal Collie and 1 normal Sheltie) that a risk assessment could not be calculated. As noted at the bottom of the table, risk assessments were only made for

genotypes observed in at least 5 dogs. However, because these dogs are not homozygous for risk alleles at either A or B (a characteristic of all high and moderate risk genotypes), this is almost certainly a low risk genotype.

Genotype	Collies		Shetland sheepdogs		Penetrance (%)	Risk*
	Cases (n = 40)	Controls (n = 185)	Cases (n = 92)	Controls (n = 205)		
<i>aabbcc</i>	0	1	0	1	-	-
<i>aabbCc</i>	0	5	0	18	-	low
<i>aabbCC</i>	1	43	1	17	-	low
<i>Aabbcc</i>	0	0	0	3	-	-
<i>AabbCc</i>	0	3	1	15	-	low
<i>AabbCC</i>	2	71	2	23	-	low
<i>aaBbcc</i>	0	0	0	5	-	low
<i>aaBbCc</i>	0	1	0	21	-	low
<i>aaBbCC</i>	0	10	0	32	-	low
<i>AaBbcc</i>	0	0	0	1	-	-
<i>AaBbCc</i>	0	0	0	14	-	low
<i>AaBbCC</i>	0	13	2	23	-	low
<i>AAbbcc</i>	1	0	0	0	-	-
<i>AAbbCc</i>	4	6	1	1	42	moderate
<i>AAbbCC</i>	15	31	7	3	39	moderate
<i>aaBBcc</i>	0	0	0	1	-	-
<i>aaBBCc</i>	0	0	0	6	-	low
<i>aaBBCC</i>	0	0	6	7	46	moderate
<i>AaBBcc</i>	0	0	2	1	-	-
<i>AaBBCc</i>	0	0	5	5	50	moderate
<i>AaBBCC</i>	3	0	23	3	90	high
<i>AABbcc</i>	0	0	3	0	-	-
<i>AABbCc</i>	0	0	2	4	33	moderate
<i>AABbCC</i>	11	1	11	1	92	high
<i>AABBcc</i>	0	0	0	0	-	-
<i>AABBcC</i>	0	0	7	0	100	high
<i>AABBCC</i>	3	0	19	0	100	high

Significant values in bold.

\*Risk interpretations were only made for three-locus genotypes observed at least five times.

One Sheltie breeder who carefully studied the above table had the following question: Since the risk for *AAbbCc* is moderate and *aaBBcC* is low, do 2 copies of the "A" allele result in dogs with a greater incidence of DMS than dogs with 2 copies of the "B" allele?

Dr. Clark's answer to the question: "We do think that A is worse than B, enough so that there is a discrepancy between the risks associated with AAbbCc (moderate) and aaBBcc (low). Unfortunately, we did not find many dogs with these genotypes."

#### Answers to Exercise # 6:

- a. This is an acceptable approach, especially since this is a new test (as of April, 2017) and very few Shelties have been tested. Yes, the risk assessment for this dog is "low", but that applies to the likelihood of him developing DMS not to his ability to produce puppies with the disease.
- b. True. *The DLA-DRB1 015:01\** allele is very uncommon. Only 7 of 297 Shelties tested [1] had that allele and the breed needs more of it! BREED that dog! 😊
- c. False. Insisting that only bitches with low risk genotypes be bred to the dog will NOT ensure that all pups will have low risk genotypes as bitches with low risk genotypes containing one or two "B" alleles, if bred to this male, would run the risk of producing moderate or high risk genotypes. (See the DMS genotype calculator or the Punnett squares.)
- d. If the stud dog owner wants to ensure that his/her dog when bred would only sire pups with low risk genotypes, this would be the way to go.

Comment: Many of you have already figured out that it is best not to double up on A's or B's, so if your dog or bitch has, for example, an "A" in its genotype, it would be best to breed it to a mate with "aa", so not to double up on the "A". The above stud dog was only low risk because the DLA-DRB1 was heterozygous.

\* The *DLA-DRB1 015:01\** allele was found primarily in Shelties from Europe, not the USA.

#### Answers to Exercise # 7:

- a) For years, Sheltie owners have used the acronym DM to refer to dermatomyositis; however, in recent years, "DM" is used to refer to degenerative myelopathy which is a disease that affects the spinal cord. (Also, there are other conditions such as diabetes mellitus and demodectic mange that may be called DM). As a result, the acronym DMS should now be used when referring to dermatomyositis. Old habits are hard to change, but be sure to use DMS from now on.
- b) The DLA numbers 003:01 and 009:01 were used in the certificates issued prior to March, 2017 and referred to DLA-**DQA1**. Since publication of the research, a decision was made to report a different set of numbers DLA-**DRB1**. The DLA-DQA1 and DLA-DRB1 genes are located very close together on chromosome 12. As a result certain alleles of these genes tend to be inherited together. In Collies and Shelties, DLA-**DQA1** 009:01 is inherited along with DLA-**DRB1** 002:01,

and DLA-**DQA1** 003:01 is inherited along with DLA-**DRB1** 023:01. The change in reporting does not change the risk interpretation.

- c) If your dog was tested before March, 2017, you should contact the Clemson lab for a new report to send to OFA as the DLA is being reported differently (as noted above) since publication of the research. Results will be unchanged. *To receive a new certificate, contact Sarah Murphy, [scmurph@g.clemson.edu](mailto:scmurph@g.clemson.edu).*
- d) Homozygous *DLA-DRB1\*002:01* means *DLA-DRB1\*002:01/002:01* (The dog received the same number from each parent, so is homozygous for the 002:01 allele.)

Heterozygous *DLA-DRB1\*002:01* means the dog inherited 2 different numbers from each parent such as *DLA-DRB1\*002:01/023:01* or *DLA-DRB1\*002:01/015:01*.

#### **Answer to Exercise # 8:**

In dogs that developed DMS, the researchers found that the more A and B risk alleles, the younger the age of onset of the disease. "Dogs having four risk alleles developed DMS at a significantly younger median age (5 months) than did dogs with only two risk alleles (18.5 months). The complete penetrance of AABB genotypes, combined with an early age of onset, suggests that these dogs may be hypersensitive to commonplace environmental stimuli (e.g., routine puppy vaccinations)." [1] The most common genotypes of affected dogs were AaBB followed by AA**bb**, AAB**b**, and AABB, all with homozygous *DLA-DRB1* of *002:01/002:01*. All affected dogs had at least two risk alleles, and all but one were homozygous for at least one risk allele. The data suggest that dogs with more risk alleles may be more likely to develop the disease at an earlier age. Also, recall that onset of DMS follows an environmental trigger, which is a stressor that dog experiences. Dogs with high or moderate risk genotypes that experience this trigger later in life will have a later age of onset, and vice-versa.

#### **Answer to Exercise #9:**

The genotype, *DLA-DRB1\* 002:01/002:01* AaBB is high risk for the dog developing DMS lesions. In the research publication, 90% of Shelties with this genotype had confirmed DMS. Only 3 of 26 were considered to be normal. [1]

This dog could be used in a breeding program by breeding him to bitches with aabb genotype. **All** puppies resulting from such matings would have low risk genotypes of aaBb or AaBb. This test allows us to maintain genetic diversity and keep desirable phenotypic qualities of dogs while decreasing the incidence of dogs with clinical signs of DMS.

**Answer to Exercise #10:**

As of June 6, 2017, there were 15. As of September 16, 2018, there were 459. Of course, the number will increase with time.

**Answer to Exercise #11:**

- Each parent has at least one copy of DLA 002:01 allele and one copy of the “B” allele since the offspring tested has 2 copies of each (one from each parent). Therefore, the above dog is homozygous for *DLA-DRB1\*002:01* and the “B” allele.
- One parent has at least one copy of the “A” allele and the other has at least one copy of the “a” allele. Since the above dog has 2 different versions of the “A” allele (Aa), it is heterozygous for it.

**Answer to Exercise #12:**

a) TRUE – Unfortunately, this genotype doesn’t tell you much about whether or not your dog will develop DMS. But, now that you know there is moderate risk, you can be more alert and responsive to hair loss around the eyes.

b) FALSE – This dog can absolutely be used in a strong breeding program. In fact, this dog carries 023:01 and this is an allele that is important to pass on and preserve in the breed.

c) TRUE – Mates would ideally not carry an A allele because the puppies could receive A from both parents and as a result themselves have moderate risk genotypes, or high risk genotypes if the mate also carries B.

**Answer to Exercise #13:**

All are true! Sometimes the trigger is not obvious. Evans et al. [1] suggest that the more risk alleles that a dog carries for DMS, the less stressful the trigger needs to be, such that something not out of the ordinary like a trip to the vet for vaccinations or a scuffle with another dog may be sufficient for disease development.

[1] From: Evans JM, Noorai RE, Tsai KL, Starr-Moss AN, Hill CM, Anderson KJ, et al. (2017) Beyond the MHC: A canine model of dermatomyositis shows a complex pattern of genetic risk involving novel loci. *PLoS Genet* 13(2): e1006604. doi:10.1371/journal.pgen.1006604.

<http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1006604#abstract1>