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Review of Dr. Henthorn's research into Cystinuria and the Non-Type 1 Cystinuria Marker DNA Test for Mastiff-Type Breeds

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Background Facts:

Human Cystinuria: Many mutations have been identified in two genes specific for kidney amino acid transport causing human cystinuria. The two genes are SLC3A1 (human chromosome 2p21), and SLC7A9 (human chromosome 19q12). The products of these two genes interact for normal renal amino acid transport and reabsorption. Defects in this system cause cystine and other amino acids to build up in the urine and can precipitate cystine stone formation. 70% of all human cystinuria cases involve mutations in one of these two genes. Over 130 novel mutations in SLC3A1, and over 100 novel mutations in SLC7A9 have been identified in human families causing clinical cystinuria. (Eggerman et. el. 2012 Orphanet J Rare Dis. 7:19) Cystinuria is typically classified as Type I if it involves an autosomal recessive mode of inheritance, and non-Type I if showing an incompletely dominant mode of inheritance where heterozygotes can be affected.

Other Breed Cystinuria: Newfoundland dogs have an autosomal recessive disorder of early age (as early as 4-6 months) cystinuria where they form stones. Dr. Henthorn discovered a (C-to-T substitution in exon 2) mutation in the SLC3A1 gene on canine chromosome 10 causing Type I cystinuria in the breed, and developed a direct genetic test for the mutation. Homozygous affected females can have cystinuria, positive NP tests, and form stones; however urinary obstruction from stones occurs primarily only in males. (Henthorn et. al. 2000 Hum Genet 107:295-303)

Current facts on Mastiff Cystinuria:

Mastiffs have a breed-related disorder of cystine stone formation, which is reported to occur only in males.

The frequency of cystine stone formation in the breed is not determined. It is reported at a frequency of 1.9% of males in the OFA On-line Mastiff Health Survey.

The earliest age of cystine stone formation in Mastiffs is approximately 12 months of age, and dogs have been identified that have blocked for the first time as late as 9 years of age.

The nitroprusside (NP) urine test is a phenotypic test for the disulfide bonds associated with cystine buildup in the urine. Abnormal NP tests are reported by UPenn at a frequency of 8.9% of Mastiff males. No females have had a verified abnormal NP test.

Many male Mastiffs can have intermittently positive NP tests, and some breeders feel that this can vary based on the level or type of proteins in the dog's diet.

All male cystine stone-forming Mastiffs studied at UPenn have at one time had a positive (abnormal) NP test (if tested when intact), showing a positive correlation between the NP test and stone formation. However, only a portion of NP positive testing Mastiffs go on to form stones.

Recent Research Findings in Dr. Henthorn's laboratory:

Based on years of testing and following serial NP test results, Dr. Henthorn has concluded that:

1) Mastiffs must be male, intact, and sexually mature to have a positive NP test, or to develop cystine stones.

2) Neutering male Mastiffs who have had a positive NP test, or who have formed cystine stones will cause them to no longer have a positive NP test, and presumably they will not form cystine stones in the future.

Additional studies following a Genome Wide Association Study (GWAS) on Mastiff DNA samples identified a region of interest. Using a series of linked markers in this region, Dr. Henthorn has developed a haplotype linked-marker test that segregates with intact male Mastiffs who have positive NP tests, or have formed cystine stones.

Labeling the abnormal marker allele 2, and the normal marker allele 1, the following trends were identified based on DNA analysis of approximately 100 intact male dogs over two years of age:

Genotypically 2-2 intact males all formed cystine stones by 4 years of age (ave. 2.5 years, range 1.5-4 years).

For genotypically 1-2 intact males, 25% formed stones at an average age of 5.5 years (range 2.5-9 years). Approximately 35% of 1-2 intact mature male dogs have had at least one positive NP test result. The remaining 40% in Dr. Henthorn's research study have not had a positive NP test result.

For genotypically 1-1 sexually mature and intact male Mastiffs, between 10%-15% have had a positive NP test result. No 1-1 testing sexually mature and intact male Mastiffs have been found to form cystine stones to date.

The samples of intact males over 2 years of age represent a skewed or biased sample set. True frequency data for the abnormal marker allele in the general population will not be able to be determined until a representative sample set from across the breed is analyzed. The current biased sample set contains approximately 20% 2-2, 35% 1-2, and 45% 1-1 genotypes.

Based on the DNA results of several Mastiff families, the linked marker genotype results are concordant with Mendelian inheritance; showing that the parental genotypes are being transmitted to the offspring as expected. (I.e., allele 2 always comes from a parent that is either 2-2 or 1-2, a 2-2 parent never has a 1-1 offspring and vise-versa, etc.) To date there has been no observed recombination between the linked marker haplotype and the occurrence of cystinuria in 2-2 dogs.

As the presence of DNA changes associated with cystinuria do not explain all cases of cystinuria (positive NP test) or completely explain cystine stone formation, Dr. Henthorn will continue to run the linked marker haplotype test on each blood or cheek swab sample, plus a NP test on urine if provided. Her research will continue to search genetic causes of the variation in NP test results and stone formation to more fully elucidate the inheritance and clinical presentation of cystinuria in the breed.

All dogs (males & females, intact or altered) should have urine sent with their samples. The $140 test fee covers both the DNA analysis and NP test. Owners should include information of sex, age, neuter status, age at neutering, and diet with all samples. Owners should notify Dr. Henthorn of any dogs that are confirmed to form cystine stones.

Comments by Dr. Bell:

We are at a point of molecular genetic discovery where the cause and clinical progression of complexly inherited disorders are being studied and elucidated. With cystine stones being limited to male Mastiffs, and with only males having positive NP test results; previous hypotheses were advanced that included X-linked inheritance, and anatomical differences between the sexes. The fact that neutering alters the ability to have a positive NP test result suggests a more complex metabolic pathogenesis to cystinuria in the breed.

Dr. Henthorn presents a scientifically sound hypothesis for cystinuria in the breed that involves the interaction of testosterone or other testicular compounds on the expression of the mutated kidney amino acid transporter molecule. There is molecular evidence to suggest a sex hormone influence on the function of kidney transporters.

Dr. Henthorn's results are consistent and concordant with a dose effect for the region of interest causing homozygous (2-2) individuals to have a more completely penetrant, and earlier onset stone formation than heterozygous (1-2) individuals. This is consistent with non-type I cystinuria.

Further research is needed to identify additional factors that cause differences in stone-forming versus non-stone-forming Mastiffs carrying allele 2, as well as NP positive males with the 1-1 genotype. Additionally, kidney samples for RNA expression studies are needed. These samples can easily be obtained by ultrasound guided percutaneous Tru-cut biopsy at the time of neutering, or during a spay procedure. This is a low risk procedure in experienced hands. Only a few dogs will be needed for this important aspect of the research. Further comparative research into cystinuria in other breeds is also indicated.

Recommendations for the use of the linked-marker test for Cystinuria:

The established cystinuria risk factor for carrying allele 2 has been satisfactorily demonstrated, and the linked-marker test can be used as a screening test for susceptibility to forming stones to determine medical management, as well as to plan matings.

Breeders must be cautioned that the genetic test is not a test for cystinuria or stone formation, but for a susceptibility allele that provides high risk in the homozygous state in intact male dogs, and lower risk in the heterozygous state.

Care will have to be taken to not significantly negatively impact the genetic diversity of the Mastiff breed by immediate and wholesale elimination of all quality breeding dogs that carry allele 2. Both male and female breeding dogs should be tested, so that planned matings avoid producing 2-2 individuals.

Quality genotypically 1-2 dogs who show desirable qualities can be bred to 1-1 mates, which will produce approximately 50% 1-1, and 50% 1-2 offspring. Quality 1-1 offspring should be used to replace genotypically 1-2 parents, so as to lose the risk allele without losing the other quality genes of the line.

By breeding and replacing quality carriers of the cystinuria susceptibility allele 2, you are replacing, and not eliminating family lines in the gene pool. Males that are 2-2 or 1-2 should be monitored for NP positive test results, stone formation, and neutered once they are no longer being shown or used for breeding. The collection of frozen semen prior to neutering can conserve the sperm of quality dogs who are at high risk of developing cystine stones.

If scientifically supported, dietary recommendations (which are beyond my expertise) for intact male 2-2 and 1-2 dogs and other NP+ dogs to minimize the risk of developing cystine stones may be an option in the future. The breed should consult with Boarded veterinary nutritionists in this pursuit.

Additional details, including the science and clinical implications behind the test as well as questions and answers, can be found in Dr. Hentorn's FAQ article on Non-Type I Cystinuria Marker DNA Test for Mastiff-Type Breeds at: http://www.mastiff.org/images/CystNonTypeIQA2013Jan04.pdf