An Overview of the Backcross Project – Part Two

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This article continues the discussion of the Backcross project begun in the preceding issue. In the first part the Dalmatian uric acid defect was introduced, the genetic mode of transmission explained, and testing procedures were examined.

How one validates that the Dalmatian uric acid defect has been corrected.

To reiterate: the genetic defect being addressed in the Backcross project is the high urinary uric acid excretion which predisposes Dalmatians to urate and uric acid stones.

– Non-Dalmatian normal uric acid excretion (10-60 mg of uric acid per day)

– Dalmatian range for uric acid excretion (400-600 mg of uric acid per day)

Data exist that place urinary uric acid concentrations for the low-UUA Backcross Dalmatian class in the same range as urinary uric acid samples taken from healthy Beagles. However, without correcting for diurnal variations (which are influenced by feeding patterns and metabolism of their diets), direct comparisons of the uric acid concentration samples for Beagles and the Backcross Dals are not possible using the existing uric acid data sets.

In this regard I am referring to quantitative assessments of urinary uric acid excretions. I do not question the legitimacy of the UUA:CR ratios as a discriminant for classifying the puppies from the Backcross litters according to whether or not they carry the normal UUA Pointer gene.

Although the 24-hour uric acid excretion values were used by Trimble and Keeler (1938) and have been discussed in Osborne and Finco (1995), they suffer to some degree from the same problem as the UUA:CR sample data. That is they do not reveal peak daily uric acid concentrations, nor do they account for the long-term, monthly fluctuations in uric acid excretion levels. However, from a purely practical standpoint, the 24-hour uric acid excretion values for a set of Backcross Dalmatians can be informative.

Without outlining a protocol, I believe that a limited set of 24-hour tests is appropriate and desirable at some time for validating the Backcross project.

Current status of the Backcross project and location of the defective gene in the Dalmatian genome.

This topic is the probably the most fascinating of any covered in this paper. First, I want to refer to a recent publication:

Mammalian Genome. 2006 Apr; 17(4):340-5. Epub 2006. Linkage analysis with an interbreed backcross maps Dalmatian hyperuricosuria to CFA03., Safra N, Schaible RH, Bannasch DL.[1]

Dalmatians, like humans, excrete uric acid in their urine. All other dogs and most mammals excrete allantoin, a water-soluble compound that is further along the purine degradation pathway. Excretion of uric acid at high concentrations (hyperuricosuria) predisposes Dalmatians to the formation of urinary urate calculi. Hyperuricosuria (huu) is found in all Dalmatians tested and is inherited as an autosomal recessive trait. A genome scan and linkage analysis performed on a Dalmatian x Pointer interbreed backcross detected a single linked marker, REN153P03, located on CFA03. Haplotype analysis of the region around this marker defined a 3.3-Mb interval flanked by single recombination events. This interval, which contains the huu mutation, is estimated to include 24 genes.

A team of geneticists at UC Davis and Dr. Bob Schaible have successfully narrowed the search for the defective UUA Dalmatian gene to a fairly small region containing only about 24 genes on canine chromosome 3. (Dogs have 39 chromosomes with a total of about 30,000 genes.) So the search for the defective gene is rapidly closing in on its quarry.

A marker on that chromosome, REN153P03, is close enough to the actual gene so that the marker can be used to flag the presence or absence of the normal UUA gene. If curious, you can consult the referenced web page to locate the marker map for canine chromosome 3 (CFA03).[2] You will find the marker, REN153P03, in the lower (magenta) depiction on that map.

A lot of information can be found both on the web and in various publications about the use of markers to aid the breeder in the selection of dogs for breeding and the elimination of hereditary diseases. A good foundational book on the subject is the AKCCHF publication, *Future Dog, Breeding for Genetic Soundness*, 1999, by Patricia J. Wilkie.

The significance of the REN153P03 marker to the Backcross project is that its use allows a breeder to identify Dals that carry the normal UUA gene by DNA analysis. Urinary uric acid excretion tests still remain as a valuable alternative. However, the DNA test has the advantage of distinguishing between a carrier and a homozygous normal condition.

How well do the DNA tests correlate with the UUA:CR tests? One of the investigators at UC Davis states: "We have never encountered a discrepancy between our [DNA] molecular testing and these phenotypes (low or high uric acid/creatinine)." So the correlation to date is perfect. Markers do not always give perfect correlations unless they lie very close to the defective gene, so this is encouraging.

Summing up the progress on the project:

- The normal UUA gene from the Pointer has been successfully integrated into the genome of the Dalmatian Backcross line.

- The Dalmatian defective UUA gene, as expected, behaves like an autosomal recessive gene as reported by Trimble and Keeler in 1938.

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- The UUA:CR ratio test unambiguously discriminates between puppies that are carriers for the Pointer gene and those that do not carry the gene.

- A gene marker has been located on canine chromosome 3 that can be used to identify Dals that carry the normal UUA gene and will also be able to identify Dals that are homozygous for that gene when they are produced.

- The gene marker, REN153P03, correlates perfectly with the classifications of Backcross puppies based on the UUA:CR ratio test.

Alternative approaches for dealing with the uric acid defect:

In this section, I outline other approaches for solving the Dalmatian urate stone problem.

1. Care and management

This approach essentially maintains the status quo. Recommended methods for minimizing the risk for urinary stones include (1) adequate hydration (there is some evidence that bottled, especially distilled, water can be beneficial), (2) provide ample opportunities for urination, (3) limit purine intake, (3) use pH test strips to monitor urine acidity, (4) use allopurinol under veterinary supervision to prevent recurrence. These basic Dalmatian husbandry procedures can help, but the underlying hereditary defect of high UUA excretion remains. Periods of stress, especially when accompanied by chronic diarrhea, can lead to acidification of urine and the formation of uric acid stones.[3] Further, unless uric acid levels are carefully monitored, treatment of stone-forming Dals with allopurinol can result in the formation of xanthine stones.[4]

2. Gene implantation

Now that the gene that causes the high UUA excretion in Dals is in the boresight of the researchers, some have suggested that high-tech gene splicing should be able to solve the Dalmatian defect without resorting to a crossbreeding to install the normal gene. Perhaps some day, but not in the foreseeable future.

Gene transfer can be targeted to somatic (body) or germ (egg and sperm) cells. In somatic gene transfer the recipient's genome is changed, but the change is not passed on to the next generation. In germline gene transfer, the parents' egg and sperm cells are changed with the goal of passing on the changes to their offspring.

Obviously, Dal breeders want to use germline gene transfer since they want the normal gene that is implanted to be passed on to the puppies.

Germline gene transfer is not being actively investigated, at least in larger animals and humans, although a great deal of discussion is being conducted about its value and desirability...[5]

There are so many intrinsic technical difficulties and risks associated with germline gene transfer that the process does not offer a practical alternative at this time.

3. Selective breeding

It has been suggested that even though all Dals excrete high levels of uric acid, not all form stones. Therefore factors other than the uric acid defect must be involved. The reasoning continues: If breed lines that consistently produce Dals that do not form stones can be identified and the environmental and subsidiary genetic factors that mitigate the stone problem understood, then selective breeding should produce stone-free Dals. Although appealing at first, there are serious problems with this line of reasoning. First, we should note that environmental factors, which were briefly mentioned under 1., above, are not refined by selective breeding. If selective breeding is to prove useful, it must deal with identifiable hereditary traits. Such traits should have a reasonably high heritability to be amenable to artificial selection methods. Further, it must be possible to identify dogs that carry the desirable traits during the period of their lives that they are used for breeding. Unfortunately, the trait *does not form stones* can only be assigned with certainty after the dog's death. Second, we should recognize, particularly in light of Dr. Susanne Hughes ultrasound study, that the category *does not form stones* might be better classified as *has not yet formed stones.* Nonetheless, recent studies with both Dalmatians and humans have identified a number of substances commonly found in urine that are known to inhibit crystal formation and the growth of urinary stones. One of the most studied is the naturally occurring Tamm-Horsfall protein which is a mucoid material produced in the kidneys. This protein is also known to provide some protection against bacterial infection in the urinary tract. Carvalho M. and others at the University of Chicago found that the amounts of Tamm-Horsfall protein (THP), and glycosaminoglycans (GAGs) were lower in Dalmatians that formed stones when compared with Dalmatians that did not form stones.[6]

If it can be demonstrated that the gene for the Tam-Horsfall protein in Dalmatians has several variants, and if the correlation between these genetic variants and the stone-forming status of Dalmatians is shown to be high, then selective breeding might help to minimize the stone-forming proclivity of Dalmatians in a carefully selected breeding line. A cautionary note must be inserted. Inhibitors of urinary stone formation do not prevent crystals from being formed and growing. These inhibitors only increase the uric acid concentrations that are maintained in solution before saturation occurs and crystals are produced -- that is the reason they are called *inhibitors* rather than *preventatives.*

This concludes an abbreviated foray into the rationale for and science behind the Backcross project. I confess to being attracted to the idea of finally doing something positive and of lasting value for the genetic health of our popular canine breed. I recall reading an article by Stephen Budiansky's "The Truth about Dogs" which was published in *The Atlantic Monthly*. The author of that article chided dog breeders for their emphasis on breed purity rather than genetic health. Budiansky recognized that not every breed carries the same defects, and damage that has been done can be undone. (*The Atlantic Monthly*; July 1999; The Truth About Dogs – 99.07; Volume 284, No. 1; page 39-53.)

The sheer diversity of dog breeds, and the fact that up until a hundred years ago -- a blink of an eye in terms of evolutionary time scales -- genes flowed freely throughout the global dog population, together imply that we still have ample genetic reserves that can be drawn on to undo any damage recently done.

If we make the effort, I believe we can make a difference.

Reference URLs:

1. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=3059479&dopt=Abstract
2. http://research.nhgri.nih.gov/dog_genome /guyon2003/guyonmaps_data/cfa03.pdf
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