

**RESEARCH PROJECT MANAGED BY DCAF**  
*Studies on Urate Urothiasis in Dalmatians*

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**Abstract:** Urinary stone disease occurs commonly in Dalmatians and is most often composed of uric acid (urate). Dalmatians have a higher risk for forming urate stones because they metabolize uric acid differently than most other breeds of dogs. Urate stones may be dissolved using special diets and a drug, called allopurinol; however, success is variable, and urate stones are highly recurrent. One problem with urate stones is that they are not usually visible on plain x-rays, which means they may be missed. Some believe many Dalmatians form urate stones in their kidneys, which may be associated with recurrent stone formation, abdominal pain, and possible kidney failure. The proposed study will evaluate two issues: 1) how commonly urate stones occur in kidneys from Dalmatians, and, 2) how effective preventative measures are for urate stones in Dalmatians.

**Final Report: Research Aims to Identify Association Among Urinary Stone Formers**

Most Dalmatian breeders and owners recognize the signs of urinary stones. Dogs suffering with stones have difficulty urinating. When they produce urine, it appears dark and thick like honey. Extreme dehydration is common as well.

About 10 percent of Dalmatians form stones even though all Dalmatians are genetically predisposed to the potentially fatal and painful condition. Researchers at the University of Tennessee College of Veterinary Medicine are trying to learn why some Dalmatians form stones and others do not. Their research, which is sponsored by the Dalmatian Club of America Foundation, involves evaluating male sibling pairs in which one dog is a known stone former and the other is a known non-stone former.

“The original Dalmatian is considered a high uric-acid (HUA) excreter, although now there are backcross Dalmatians known as low uric-acid (LUA) excreters,” explains Joseph Bartges, D.V.M., Ph.D., DACVIM, DACVN, professor of medicine and nutrition and the lead investigator. “If all HUA Dalmatians excrete high levels of uric acid in their urine, why don’t they all form urate stones? There must be something else that separates a urate-urolith forming Dalmatian from non-urate-urolith forming Dalmatians.”

Genetic researchers identified a mutation in the SLC2A9 gene in Dalmatians that causes hyperuricosuria, or high uric acid levels, in 2009. Danika Bannasch, D.V.M., associate professor of veterinary genetics at the University of California, Davis, and Bob Schaible from Purdue University, analyzed urine and DNA samples from a backcrossed line of Dalmatians and a single Pointer.

While all Dalmatians are homozygous for the gene mutation, meaning they inherit two copies of the mutation, the backcross dogs were heterozygous, or had one normal copy of the gene. The backcross dogs, though they had a similar genetic makeup to the Dalmatians, produced normal levels of uric acid.

The SLC2A9 gene mutation results in an amino acid deficiency of a key protein involved in transporting uric acid to the liver. This contributes to increased excretion of uric acid and affects metabolism of uric acid into allantoin. While most mammals are able to metabolize

purine compounds and produce water-soluble allantoin as a waste product, Dalmatians along with humans, great apes and English Bulldogs produce a different breakdown product, uric acid. Elevated uric acid levels can result in the production of uric acid salts or urate that crystallize and form stones.

Bartges' research is investigating whether the gene mutation alone accounts for a predisposition to form uroliths. "We feel other factors are involved," he says. "Our genomewide comparison of littermates has identified several other genomic differences that may be involved in urolith formation."

Urinary stones can form at any age. If not treated immediately, stones can lead to urinary blockage. Males tend to obstruct because their urinary pathway passes over the os penis where stones can get lodged and cause an obstruction. Females rarely obstruct because there is no narrowing of the urinary pathway. The obstruction occurs when no urine is passing or when very little urine can pass.

### **A Possible Kidney Association**

One challenge hampering understanding Dalmatian stone disease is that the stones may not be visible on standard radiographs. The inability to visualize stones means it is not always clear which part of the urinary system -- the bladder or kidneys -- they originated. The majority of urinary stones are believed to occur in the bladder.

Stone disease may advance to kidney insufficiency and subsequent kidney failure. "Some people believe many Dalmatians form urate stones in their kidneys, which may be associated with recurrent stone formation, abdominal pain and possible kidney failure," Bartges explains. "Our study will evaluate how commonly urate stones form in the kidneys and the effectiveness of preventive measures for urate stones. We want to know whether stone formation also takes place in the kidneys."

A hypothesis is that if urinary stones originate in the kidneys, kidney complications may be reversible by using effective anti-stone therapies that target the upper urinary system where the kidneys are located. The findings potentially will benefit Dalmatians with stone disease by helping to extend their lives and avoiding costly expenses for kidney insufficiency disease or kidney transplants.

Bartges has been involved with urolith research for 25 years, having written his doctoral dissertation on uric acid metabolism and urate stones. A component of this research involves collecting autopsy information from the kidneys and ureters of stone-forming Dalmatians. It is the first scientific project to gather autopsy data on Dalmatian stone disease in the kidneys and upper urinary system.

"There is concern about the number of Dalmatians diagnosed with kidney failure and/or kidney insufficiency," says Bartges. "Some cases of these life-threatening diseases may be caused by undetected stone formation in the kidneys and the upper urinary system. If this is so, there are known anti-stone treatments that may benefit both conditions."

The number of Dalmatians affected by urinary stones is not known. Some reports indicate as many as one-quarter to one-third of Dalmatians develop stones, while other sources suggest the disorder affects from 5 to 10 percent of the breed.

In their study, Bartges and his team have conducted a genomic evaluation of 20 male sibling pairs. They plan to evaluate 10 more pairs. In each pair, one dog was a known stone former and the other one was a known non-stone former. Males were only evaluated because

they are more predisposed than females and because they are more easily diagnosed. Additionally, it takes out gender-associated differences.

Conducting a genomewide analysis, with help from a biostatistician, they are looking for associations between the two groups that will help to explain a genetic basis for stone formation. They evaluated 147,000 SNPs (single nucleotide polymorphisms) and found 874 that were different between the stone formers and the non-stone formers. Of these, 220 had less frequency, which gave them 654 possible associations.

“In assessing whether these had some importance, we were able to narrow it to seven strong candidate genes,” Bartges says. “Three of these were not involved in a coding region, and of these, two seemed to clinically/physiologically make sense.”

The two possible candidate genes identified are:

- SLC8A1 on chromosome 17
- CACNA1C on chromosome 27

“The SLC8A1 gene on chromosome 17 is most interesting,” he says. “Other SLC genes have been associated with high uric-acid levels. Specifically, SLC2A9 variants may be responsible for high uric-acid levels in Dalmatians, and SLC22A12 is involved with uric-acid stone diseases in humans. In a study of human familial juvenile hyperuricemic nephropathy, the SLC8A1 region was identified.

“To pursue this a bit further, we believe that we only need an additional 10 sibling pair samples or 20 non-sibling pair samples – male stone formers and male non-stone formers – to be able to determine the relevance of these findings.”

The majority of differences in the chromosomes of individual dogs are SNPs, which are a change of one nucleotide or letter in the DNA sequence. Although some SNPs have functional effects that alter the biology of any animal, the majority of SNPs have no biological significance but can be used as markers to identify the chromosomal region carrying a mutation.

The SNP chips allow the researchers to focus on a small area of the canine genome by comparing profiles of affected and healthy dogs. The regions of difference help to distinguish the location of disease genes, and then the researchers can evaluate the genes and surrounding DNA sequence.