



Hepatic Copper Storage Disorder in the Dalmatian

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Betty Garvin Memorial Lecture

<http://s3.amazonaws.com/cdn-origin-etr.akic.org/wp-content/uploads/2017/11/12234021/Dalmatian-On-White-03-400x267.jpg>

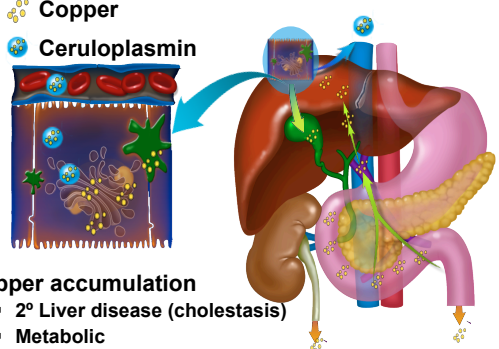
Copper Is Essential For Life



- Energy production
- Nerve transmission
- Antioxidant function
- Iron metabolism

Normal Copper Metabolism

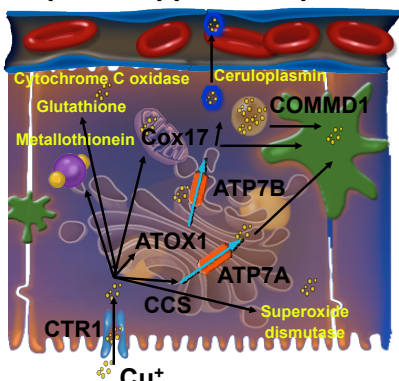
● Copper
● Ceruloplasmin



Copper accumulation

- 2° Liver disease (cholestasis)
- Metabolic
- Dietary

Hepatic Copper Transport



Cytochrome C oxidase
 Glutathione
 Metallothionein
 Cox17
 ATOX1
 CCS
 CTR1
 Cu⁺
 Ceruloplasmin
 COMMD1
 ATP7B
 ATP7A
 Superoxide dismutase

Wilson disease: Copper Storage Disease in Humans

Cirrhosis of the Liver

The diagram illustrates the clinical manifestations of Wilson disease. At the top, a liver is shown with a textured, bumpy surface, labeled 'Cirrhosis of the Liver'. Below it, a blue double-headed arrow labeled 'Copper' connects three images: a close-up of a human eye showing a Kayser-Fleischer ring (a dark ring at the periphery of the cornea), a liver, and a human head in profile with a blue glow on the brain, indicating neurological symptoms.

Wilson disease: Copper Genetics in Humans

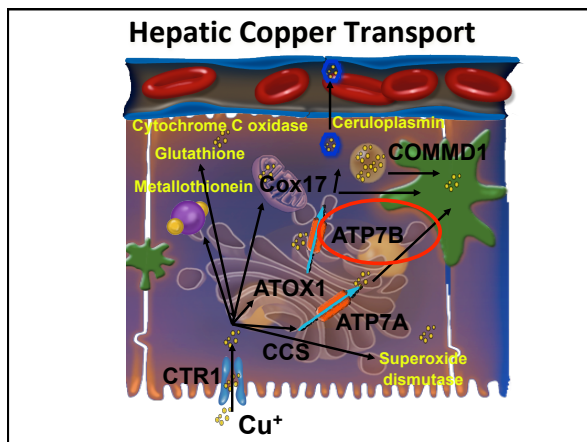
Professor emerita Diane Cox, at U Toronto

ATP7B gene discovered in 1993

Over 100 variants in the *ATP7B* gene in many discovered by Dr Cox while at U Alberta

Questions in human studies: how many of the genetic changes are 'normal' and how many cause disease?

The slide features a portrait of Professor Diane Cox on the left. To her right is a 3D ribbon diagram of the ATP7B protein structure, showing various domains and amino acid residues. The University of Alberta logo is at the bottom left.



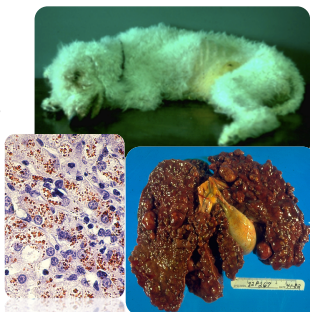
So the Canine Copper Story Begins.....

- 1975 Marianna Padula described liver problems in her Bedlington terriers
- 1976 Hardy reported in *The Minnesota Vet* abnormal copper in livers of several Bedlington terriers having liver disease

The slide includes a photograph of Marianna Padula with a dog and a microscopic image of liver cells containing numerous blue-stained copper granules. The caption below the image reads 'Copper Granules in liver Cells'.

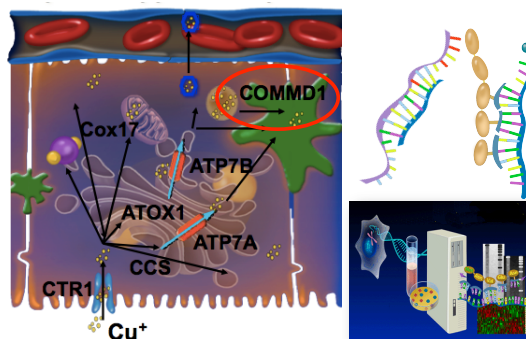
Bedlington Terrier Copper Hepatotoxicity

- Copper increases with age of dog
- Successful treatment with chelation therapy
- Genetic defect identified (COMMD1)
- Genetic testing has almost eliminated the disease



Twedt et al: JAVMA175;1979

Bedlington terrier Genetics



Breed Predispositions for Chronic Hepatitis and Copper

- Bedlington terrier
- Doberman pinscher
- Labrador retriever
- West Highland white terrier

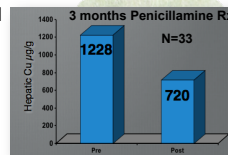


Normal Copper
120-400 µg/g dw

Toxic Levels
>1000 µg/g dw

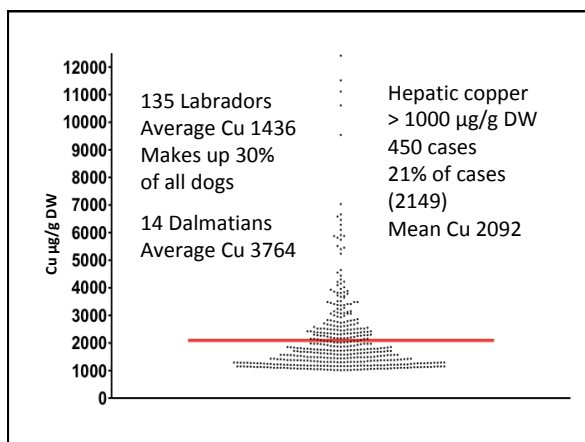
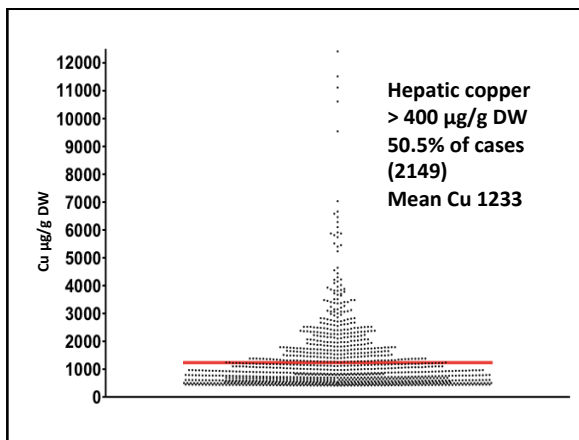
Chronic Hepatitis in Labrador Retrievers

- Hepatitis associated with hepatic Cu
- ATP7B defect
 - Same gene as in humans
 - Males protected if they carry a mutation in ATP7A
- Penicillamine reduces Cu
- High dietary Cu associated with high hepatic Cu
- Low Cu diets prevent Cu accumulation



Incidence of Abnormal Hepatic Copper in Dogs

- Reviewed CSU Diagnostic Laboratory records between 2010 – 2015 having both liver histology and liver copper quantitation ($\mu\text{g/g}$ dry weight; $N < 400$)
 - 2149 samples
 - 1064 $\text{Cu} \leq 400$
 - 1085 $\text{Cu} > 400$ (50.5%)



.....Remembering the words of one of my mentors

- “.....they put too much copper in dog foods”
- Man hepatic Cu
 - Normal 50-75 $\mu\text{g/g}$
 - Wilsons Dz $>400 \mu\text{g/g}$
- Dogs hepatic Cu
 - Normal 200-400 $\mu\text{g/g}$
 - Normal in 1930's 50-75 $\mu\text{g/g}$
- Could the advent of commercial dog food be the cause for this increase and are certain dogs unable to handle the copper in the diet?



Relevant Dog Food Characteristics

	Average Dog Food	RC Hepatic Hills l/d	AAFCO min, max	NRC minimum
Copper (mg/kg DM)	15-25	4.2-4.8	7.3 min 250 max	6

- Feeding recommendations based on assumptions (puppies and pigs)
- In 1997 dietary copper supplement switched from Cu oxide to Cu chelates
- The average 10kg dog consumes twice the copper a day than a 70 kg person

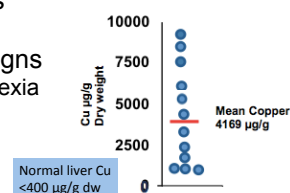
J Vet Intern Med. 2002 Nov-Dec;16(6):665-8.

Copper-associated liver disease in Dalmatians: a review of 10 dogs

Webb CB¹, Twedt DC, Meyer DJ.

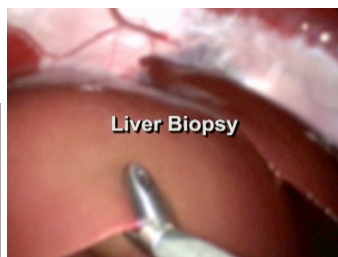
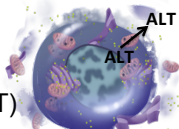


- Average age 6 yrs.
 - Range 2-10
- 4 males, 6 females
 - One mother & son
- Gastrointestinal signs
 - Vomiting and anorexia
 - Lethargy
- Icteric membrane



Diagnosis

- Abnormal liver enzymes (ALT)
- Liver biopsy:
 - Surgery
 - Needle biopsy
 - Laparoscopy



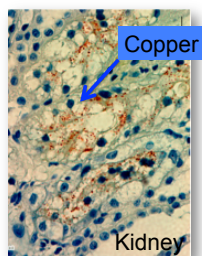
Diagnosis

- Liver biopsy:
 - Histopathology
 - Special Cu stains
 - Cu quantitation
 - 5X5mm or > diameter tissue
 - Place in Cu free container
 - It is possible to measure Cu on paraffin embedded sample (after the fact)



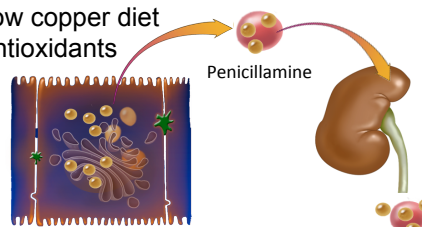
Maybe a Clue to Copper Toxicity?

- Urine sample:
 - Some may have glycosuria
 - Sugar in the urine
 - Cu associated Fanconi syndrome?
 - Cu stain in kidney tubules
 - Resolves with therapy



Treatment

- Copper chelation
 - Penicillamine
 - Others?
- Zinc (too slow acting)
- Low copper diet
- Antioxidants



Treatment

- Penicillamine (10-15 mg/kg q12 h)
 - Use compounded formulations
 - Give on empty stomach
 - May cause vomiting in some
 - Treat until ALT is normal
 - Re-biopsy is ideal
- Low copper diets
 - RC Hepatic™
 - Hills I/d™
 - Homemade diets
 - BalancelT.com
 - Vitamin supplements low in Cu



Understanding the Genetics of Hepatic Copper Toxicosis in the Dalmatian

Principal Investigator: Andrew Lawrence Mason, PhD; University of Alberta

Total Grant Amount: \$100,000

Grant Period: 3/1/2017- 2/28/2019

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CSD Gene Hunters

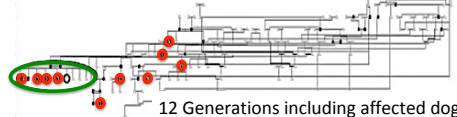
Dr. Charles Garvin
Director DCA
Foundation
Chair AKC Canine Health Foundation



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Copper Storage Disease in the Dalmatian: Our Study

- Phase I – collect and evaluate data
 - CSD Study Group provided information
 - Owners and breeders
 - Liver samples and DNA to CSU
 - Orthopedic Foundation for Animals (OFA) database for pedigree information/DNA bank
- 163 dogs on the pedigree below
- 22 biopsy confirmed dogs (19 affected, 1 carrier)



12 Generations including affected dogs

Copper Storage Disease in the Dalmatian: Our Study

- Dog has 39 pairs of chromosomes
- 19,000 protein coding genes

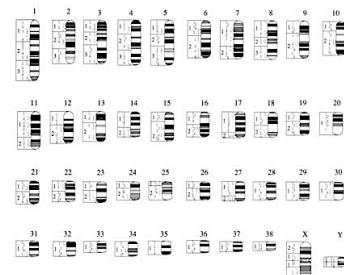
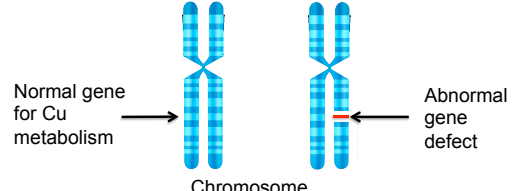


Fig. 1. Karyotype diagram of the canine karyotype with characteristic landmarks and #19 numbered bands.

Copper Storage Disease in the Dalmatian: Our Study

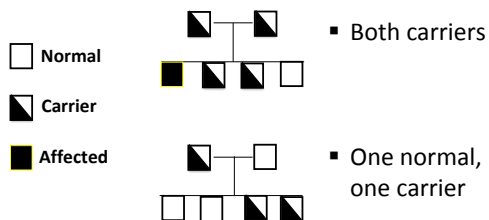
- Phase I – Preliminary results:
 - Most likely an autosomal recessive defect due to an abnormal gene involved in Cu metabolism



Normal gene for Cu metabolism → ← Abnormal gene defect

Chromosome

So what does this mean.....



Copper Storage Disease in the Dalmatian: Our Study

- Phase II –
 - DNA whole genome sequencing from select dogs
 - Identify candidate genes leading to CSD in Dalmatians
- Phase III
 - Develop genetic tests for hepatic CSD
 - Use genetic tests to reduce breeding of affected dogs
 - Use genetic tests to develop targeted therapies
 - Once the Dalmatian genome is established can lead to future studies of other disorders

Many thanks to all of the Dalmatian owners and breeders who have contributed so far!

Continued need for information from owners and breeders
 If you have provided information and/or samples in the past – please get in touch, as we need your consent to use any information previously submitted to the CDSG for this new study



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<http://s3.amazonaws.com/cdn-origin-etr.akc.org/wp-content/uploads/2017/11/12234021/Dalmatian-On-White-03-400x267.jpg>