Hepatic Copper Storage Disorder in the Dalmatian

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Betty Garvin Memorial Lecture

Copper Is Essential For Life

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

Copper Is Essential For Life

Normal Copper Metabolism

- Copper
- Ceruloplasmin

Copper accumulation
- 2nd Liver disease (cholestasis)
- Metabolic
- Dietary

Hepatic Copper Transport

- Ceruloplasmin
- Glutathione
- Metallothionein
- Superoxide dismutase
- Commd1
- Atox1
- Ctr1
- Ccs
Wilson disease: Copper Storage Disease in Humans

Cirrhosis of the Liver

Copper

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?

Hepatic Copper Transport

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

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

Tweedt 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

Twedt et al: JAVMA175;1979
Incidence of Abnormal Hepatic Copper in Dogs

- Reviewed CSU Diagnostic Laboratory records between 2010 – 2015 having both liver histology and liver copper quantitation (µg/g dry weight; N < 400)
- 2149 samples
- 1064 Cu < 400
- 1085 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 µg/g
  - Wilsons Dz >400 µg/g
- Dogs hepatic Cu
  - Normal 200-400 µg/g
  - Normal in 1930’s 50-75 µg/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

<table>
<thead>
<tr>
<th>Copper (mg/kg DM)</th>
<th>Average Dog Food</th>
<th>RC Hepatic Hills l/d</th>
<th>AAFCO min, max</th>
<th>NRC minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-25</td>
<td>5.2-4.8</td>
<td>7.3 min</td>
<td>250 max</td>
<td>6</td>
</tr>
</tbody>
</table>

- 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

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

Webb CG, Tweed 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

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 l/d™
- Homemade diets
  - BalanceIT.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
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Grant Period: 3/1/2017 - 3/26/2019

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Copper Storage Disease in the Dalmatian: Our Study

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

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

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)
So what does this mean..........

- Both carriers

- One normal, one carrier

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**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

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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 CSDSG for this new study.

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