Update from June 27, 2018: Search for the gene and mutation causing inherited cataract(s) in American cocker spaniels

July 2, 2019

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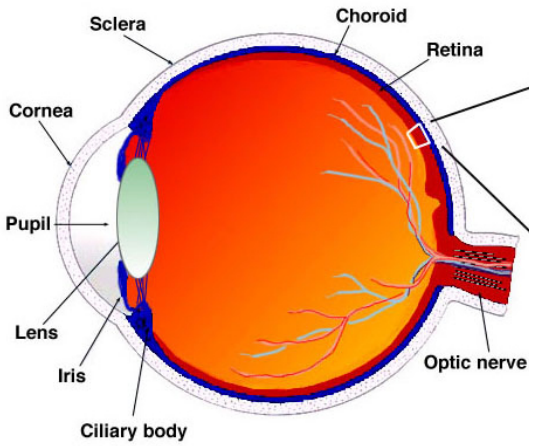
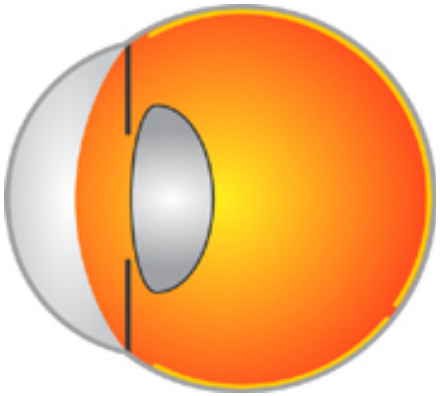
Cataract: any opacity/cloudiness of the lens

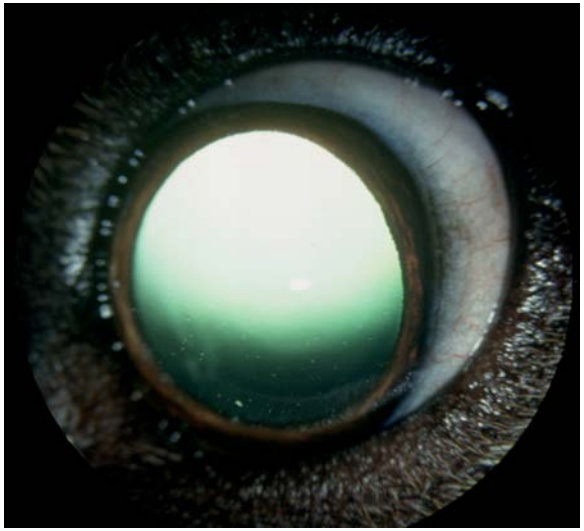
Clinical use of term:

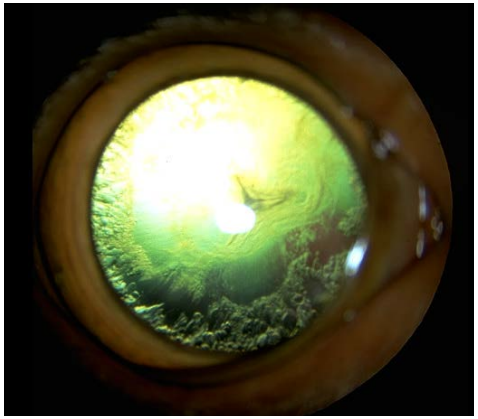
Opacity: usually means it is not significant (hereditary), and will not progress to impair vision

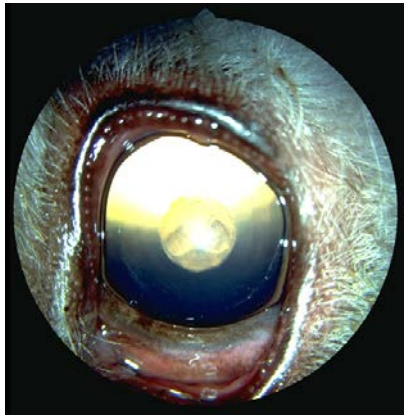
Cataract: inherited, and, depending on the type of cataract and breed, may progress and impair vision or cause blindness.











Cocker Spaniel Inherited Cataract Research Study

INSTRUCTIONS: In addition to collecting 3-5 ml of whole unclotted blood in an EDTA tube from each dog, please include:

Completed form: page 1 completed by owner.
Completed form: pages 2 & 3 completed by ophthalmologist
5-6 generation pedigree of the affected dog
Current and any/all previous eye exams on the affected dog
Fundus photographs: either printed or emailed to suepk@optigen.com
Additional blood samples from sire, dam and siblings, if available together with completed eye examination forms.

The blood and paperwork should be sent via US Mail, or a commercial shipper to OptiGen, 767 Warren Road, Suite 300, Ithaca, NY 14850. The blood vial should be protected from breakage during shipping. Suggestions for packaging are viewable on the Optigen website: http://www.optigen.com/opt9_shipsubpg3pkg.html

OWNER Information
Name: first_____ initial ____ last_____
Address:_____
City: _____ State/Province: _____
Country: _____ Zip/Postal Code: _____
Day Phone: _____ Evening Phone: _____
Fax: _____ Email: _____

DOG IDENTIFICATION (Indicate "N/A" if question not applicable)
Breed :_____ Call Name: _____
Registered Name:_____
Registration #: _____

Birthdate: ___/___/___ (mon/day/yr) Sex: ___Female ___Male

Registered Name of Sire:
_____
Registered Number of Sire:
_____
Registered Name of Dam:
_____
Registered Number of Dam:
_____

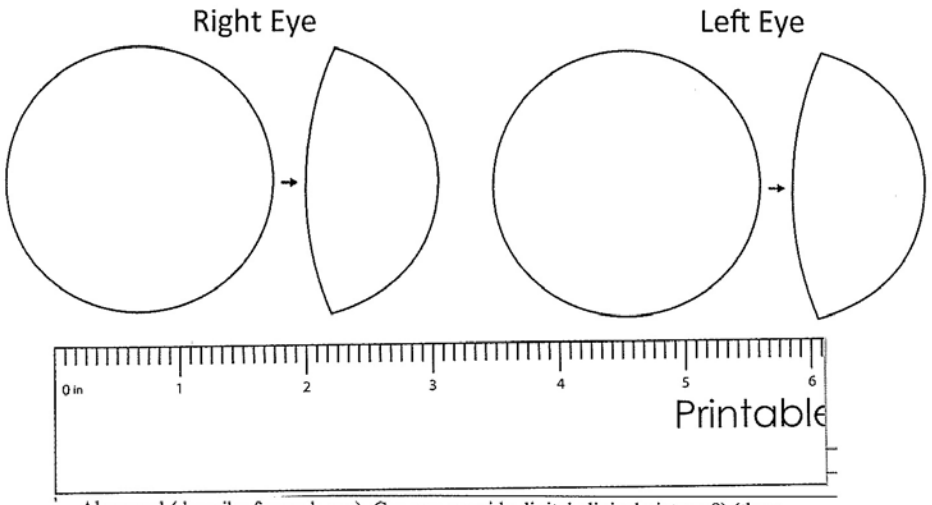
Number of full siblings of affected dog, including repeat matings of parents: _____

Are there any other cases of inherited cataracts known to have occurred in relatives of this dog? Yes_____ No_____ If yes, please describe relationship to affected dog or identify in pedigree and whether blood samples and clinical examination records are available from any of these dogs:-
_____
_____
_____

Ophthalmologist/Clinician Contact Information

Name: first_____ initial ____ last_____
Address:_____
City: _____ State/Province: _____
Country: _____ Zip/Postal Code: _____
Day Phone: _____ Evening Phone: _____
Fax: _____ Email: _____

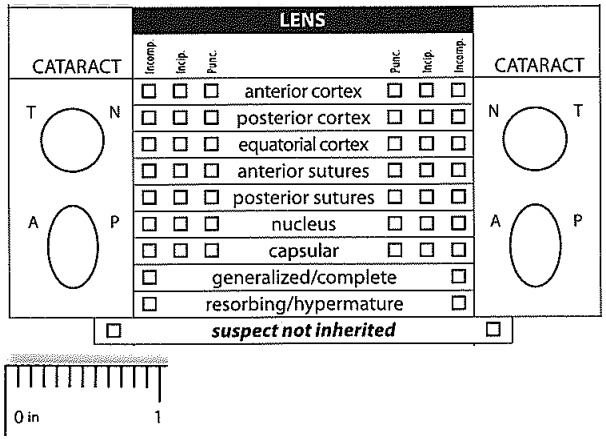
Clinical examination results (using slit lamp biomicroscope):



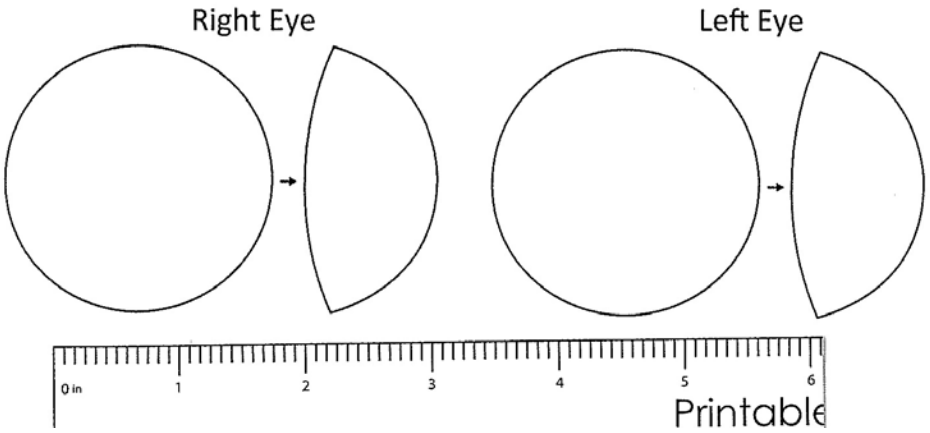
Abnormal (describe for each eye). Can you provide digital clinical pictures?) (these should be sent to Optigen,LLC)
_____
_____
_____
_____
_____
_____
_____

In your opinion, are the cataracts inherited, acquired or of unknown cause?
_____
_____
_____
_____

CAER/OFA



Cocker spaniel research form



Advantages of the research form:

- lens is anatomically correct.
- larger size of lens schematic to allow for detailed illustration of the clinical findings.
- sufficient space to allow for examiner to write comments or provide interpretation or opinion.
- some ophthalmologists, fortunately a minority, 'know better' and do not use the form and send records that are not useful for the research.

The working hypothesis has always been that the mode of inheritance is simple autosomal recessive. This has directed the initial phase of the research.

FAMILIAL CATARACTS
IN THE
AMERICAN COCKER SPANIEL

William L. Yakely, D.V.M., M.S.
G. A. Hegreberg, D.V.M., Ph.D.
G. A. Padgett, D.V.M., M.S.

J. American Animal Hospital Association, 1971

A Study of Heritability of Cataracts in the American Cocker Spaniel

3540

W. L. Yakely, DVM, MS

JAVMA, Vol 172, No. 7 **1978**

We now know that is not the case, and have redirected or efforts to examine this new interpretation of heritability of cataracts in the American cocker spaniel.

June, 2019 conclusion: *We need samples of dogs diagnosed with bilateral cortical cataracts. We are considering 2 groups of cataract affected dogs: a)-cataracts present between 2-5 yrs of age; b)-cataracts present between >5-8 yrs. To have a good sample set of affected dogs. We need at least 10-25 more "gold standard" cases that fulfill these conditions.*
We need 10-25 more "gold standard" normal dogs (normal at $\geq$ 9 years)

Between July 2017-September 2017

17 affected
30 normal

8 dogs added [the rest too young for a gold standard control, or an unreliable phenotype for being a good case (most of the exclusions)]. In other cases we did not have the DNA and we gave priority to other dogs. Estim. 6-12 additional dogs can be monitored and re-evaluated to become controls)

Between October 2017-March 2018

29 affected
13 normal

3 dogs added to study that came before 2018 . Most of these dogs are not in the archive yet and records are now being evaluated to include them.

Between April 2018 and June 2018: 115 samples

16 affected
10 normal

Total # samples by June '19 – now: **180 samples**

Affected: 52
Normal: 71
Excluded as updates accumulated: 57

Source of Imperfections

Dina Torjman has been the Research Specialist since October, 2017. Since then she has:

- reached out to **~160** people between both email and phone. Of those people, **~100 responded and were very helpful**. There were only about 3-5 people who were responsive, but *not* helpful (no knowledge that dog was even in study, no longer have the dog & either don't know who the new owners are or don't want to put me in touch with them, unwilling to bring dog to veterinary ophthalmologist, etc.)
- there are around 11 people who I have incorrect contact info for and have been unable to track down (invalid email addresses, disconnected phone numbers, exhausted google & public records…)

BUT……

- Lastly, I have received **~150 updates unsolicited, without having to ask. This has been <u>extremely helpful.</u>**

State of data as 2019

As of June 2019, we have collected 793 blood samples/records/pedigrees.

Total dogs	**793**
Total of Informative dogs	**534**
Potential cases	***93***
Bilateral	72
Unilateral or very Asymmetric	21
Controls	***441***
Too young to be properly assessed	185
Total of 'Excluded' dogs (as of this stage)	**259**

State of data as 2019

Reason for exclusion:

- Co-morbidity with another eye condition
- Doubts about diet/medications etc
- Dog prematurely deceased (especially if DNA/blood is missing)
- Lack of feedback on updates (now a **very rare** occurrence)
- Lack of an official diagnosis by a certified veterinary ophthalmologist (or of monitoring post diagnosis)
- Inconsistent records (very rare occurrence)
- Dog too young to tell (will change over time) as dogs are re-examined and enter age range needed for the study.

Further selection for the SNP genotyping:
Sufficient amount of records over time!

Our Research Approach

• collect samples from **phenotype ascertained** dogs: normal (controls) and affected with cataracts (cases).

• establish what is the minimum age when dog is considered a control.

• group cases into 'reasonable phenotype groupings.

• reconsider your groupings as number of cases comes in. Example:

-initial cases were bilateral ant/post cortical cataracts in 2-5 yr range.

-after looking at >200 dogs, and receiving close to 800 records, early grouping strategy was revised to:

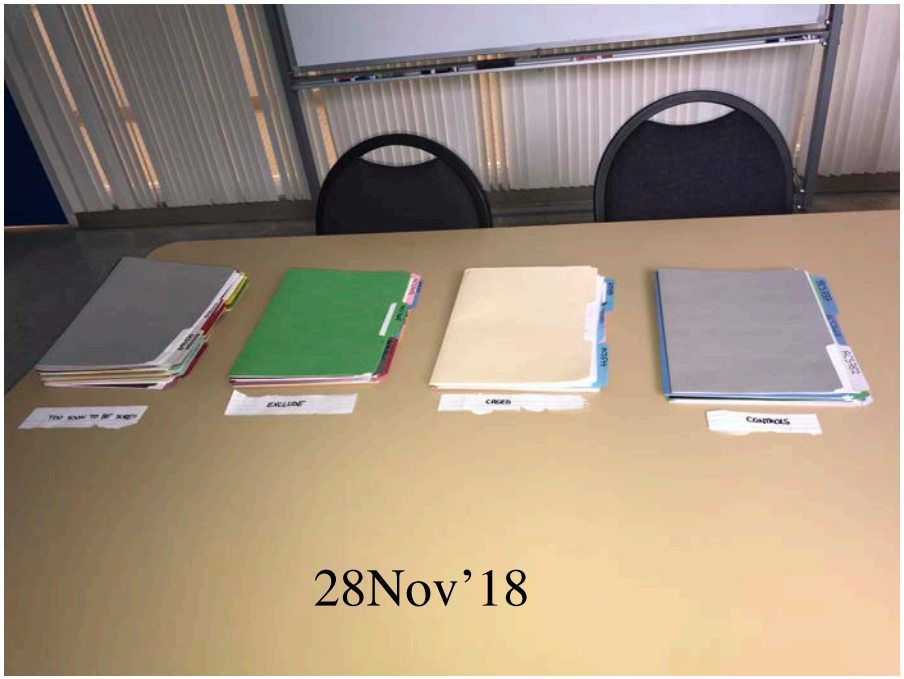
* Cataracts had to be progressive even if they "looked" inherited.
* young (2-5 yr) and older (5-8 yrs) cases need to be included.
* Unilateral or asymmetric cataract cases need to be included.

New approach evolved not from wishful thinking, but based on data, and we revised our approach depending on the study results.

• With groups, carry out Genome Wide Association Study (GWAS)

• Identify chromosomal region of interest and do Whole Genome Sequencing (WGS). **We are at this stage now !!!.**

26July'18



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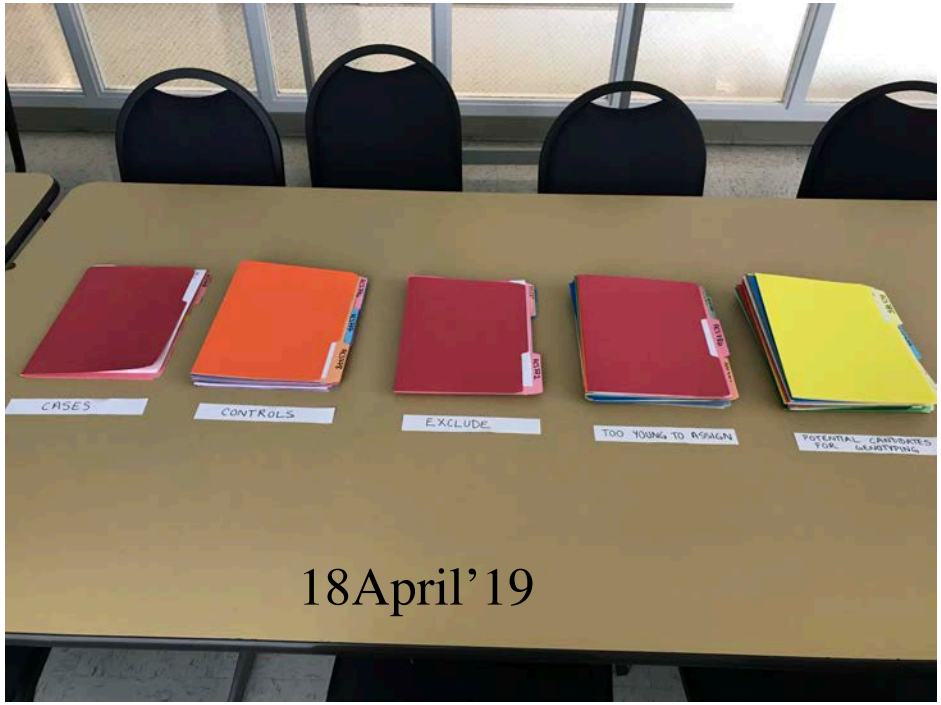


18April'19

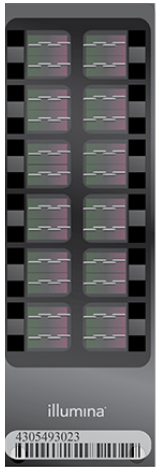
Number of samples received:

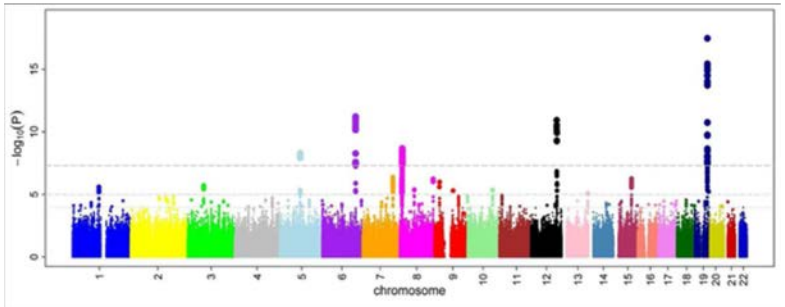
-673 as of January, 2019
(~230 examined by GDA)

Genome-Wide Association Study (GWAS)

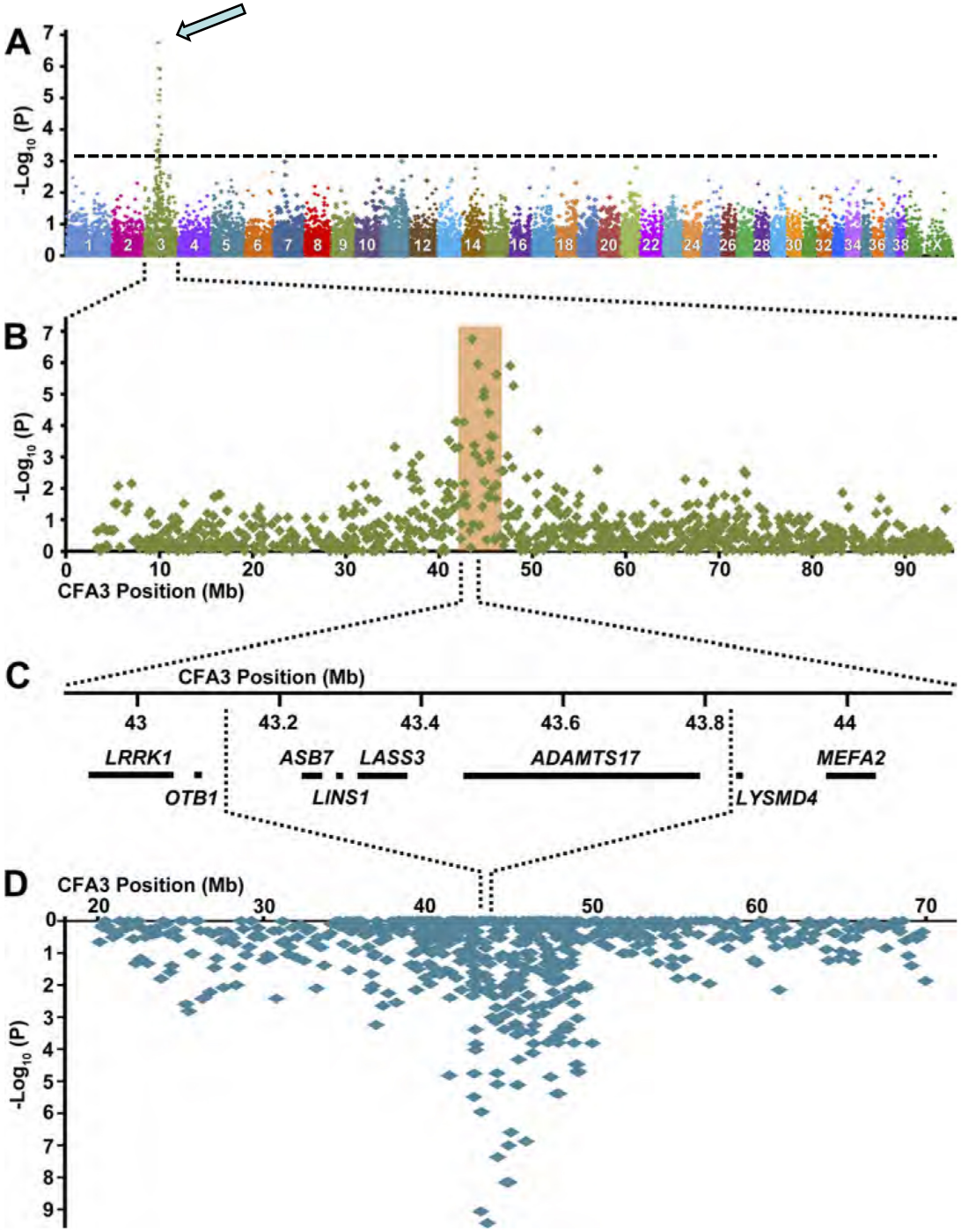
In genetics, GWAS is an observational study of a genome-wide set of genetic variants [aka single nucleotide polymorphisms (SNPs)] in different individuals to see if any variant is associated with a trait. GWAS typically focus on associations between SNPs and inherited traits (e.g. coat color, length of hair, defects**-CATARACTS**).

As long as the trait can be scored accurately in a sufficiently large population of cases and controls, the position of the trait in the genome can be localized. Then the gene/specific defect is identified.





example of a “Manhattan” plot from a human disease study (note: 22 pairs of autosomes + X/Y sex chromosomes)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CFA3 Position (Mb)
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OTB1
ASB7
LINS1
LASS3
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LYSMD4
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Samples used for GWAS

- (**'First Batch'**) Dec 2015: 48 initial samples (after analysis of clinical records, 10 cases and 10 controls eliminated)
- (**'2nd Batch'**) Feb 2016: Second batch: 21 samples added (69) - 12 excluded
- (**'3rd Batch'**)Apr 2017: Third batch: 55 samples added (124) - 6 excluded
- (**'4th Batch'**)May 2018: Fourth Batch. 37 samples added (161) - 8 excluded
- (**'5th Batch'**) Sept 2018: Fifth batch: 12 (172) – 6 excluded
- (**'6th Batch'**) Feb 2019 : Sixth batch: 7 (180) – 5 excluded

- Diagnoses routinely re-analyzed, dogs inserted and excluded from the study as necessary from the updated data

- Current dataset of 180 dogs
  - **52 cases**
  - **71 controls**
  - 57 excluded

  In addition:
  - 11 additional controls have been recently obtained ("promoted" re-examined dogs)
  - **42** dogs too young to tell or with incomplete records are soon to be re-examined

State of data as 2019

Total genotyped	
Cases*	**52**
First class (>2 – 5< yr)	27
Older age category (>5 – 8< yr)	13
Second class	12
Controls	**71**
First class	40
Second Class	17
Third class	14
Excluded	**57**

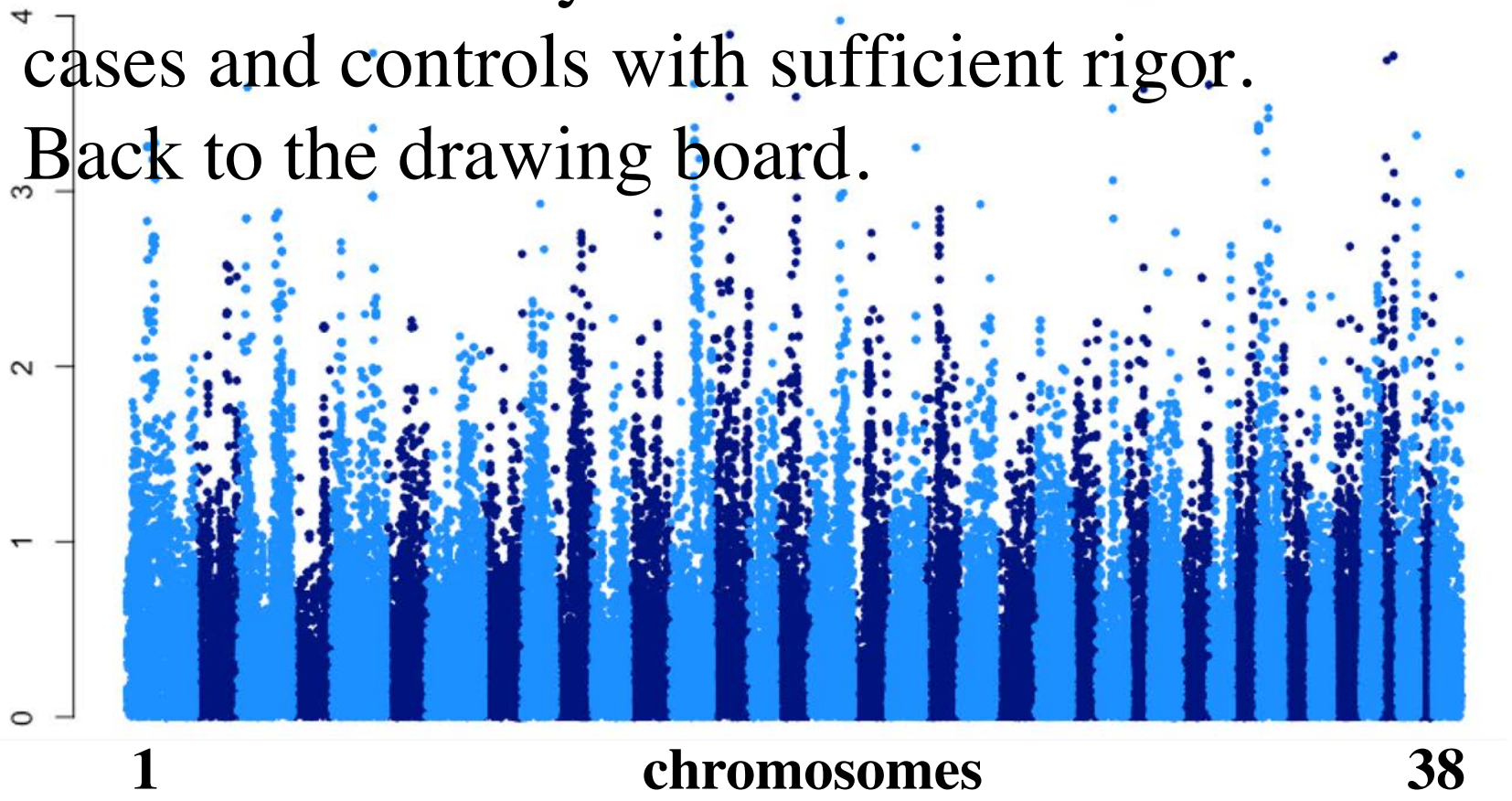
*28 bilateral, 17 asymmetrical, 5 unilateral

American cocker spaniel-cataract study
Whole population, **first** batch

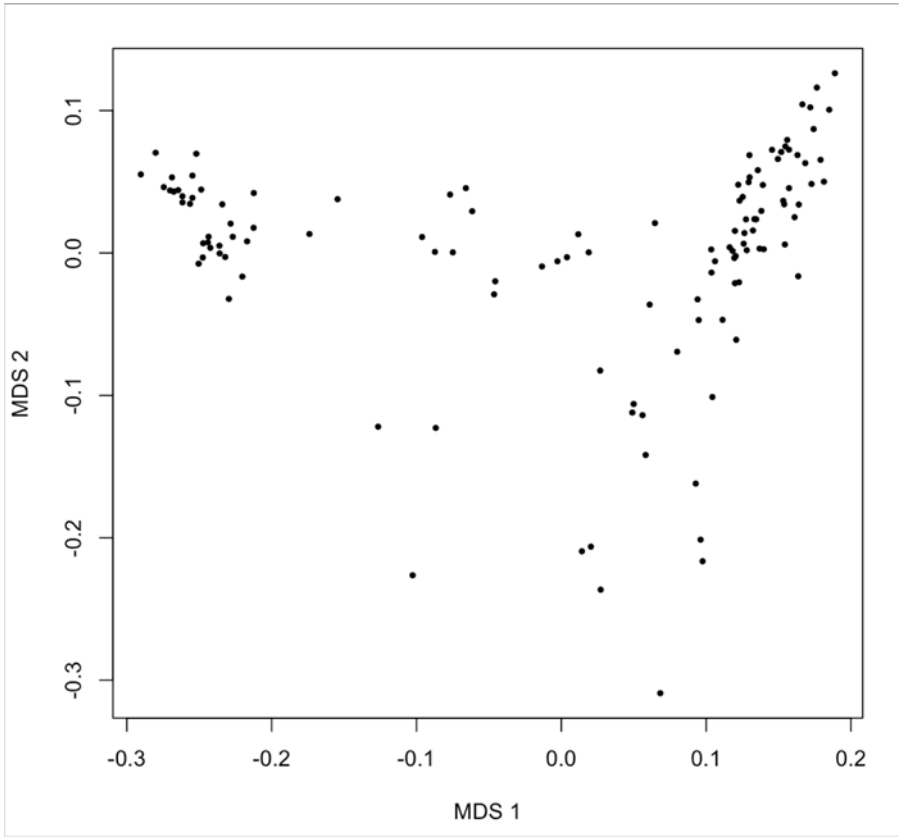
NOT good.

What does it tell you? We have not selected the cases and controls with sufficient rigor.

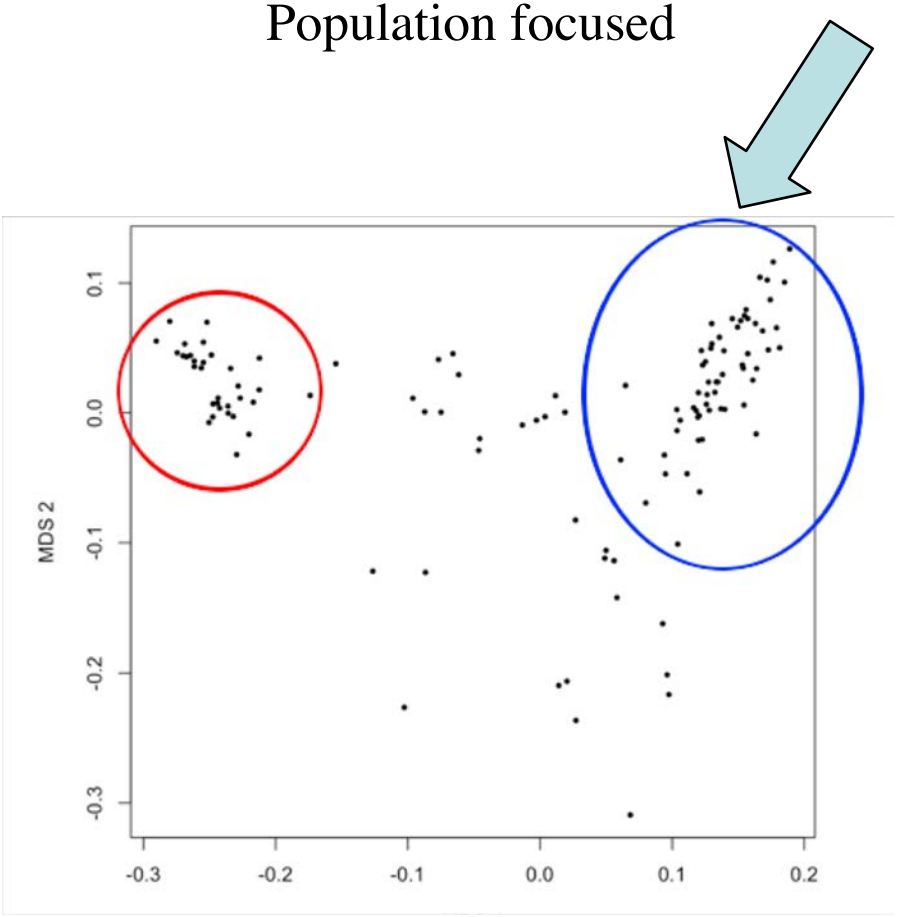
Back to the drawing board.



MDS plot

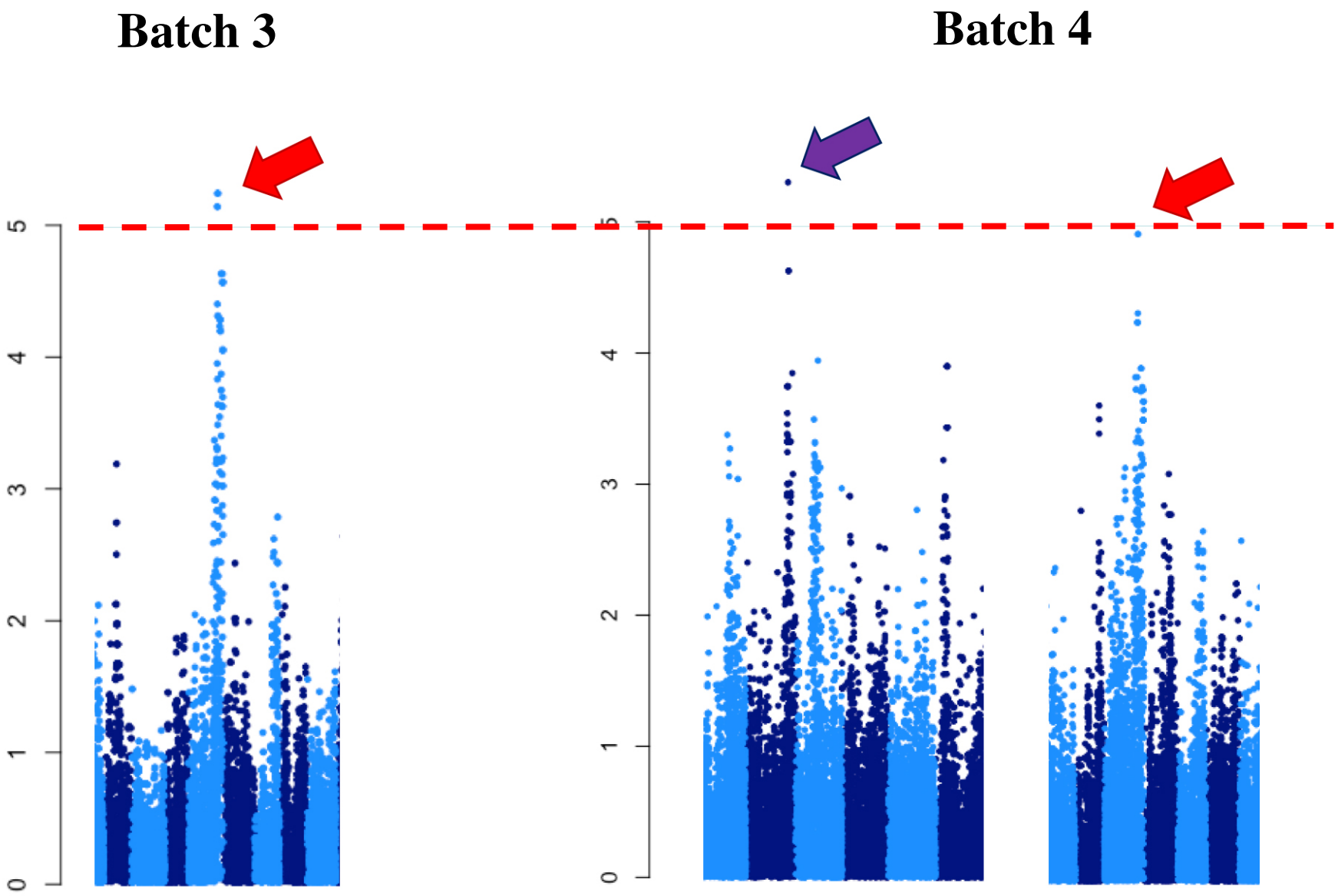


Population focused



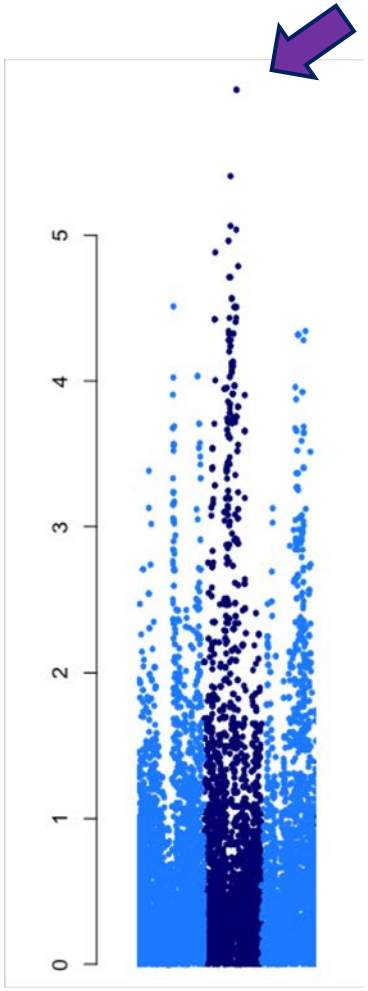
Multi**d**imensional **s**caling (**MDS**) shows the level of similarity of individual cases of a dataset.

Right population

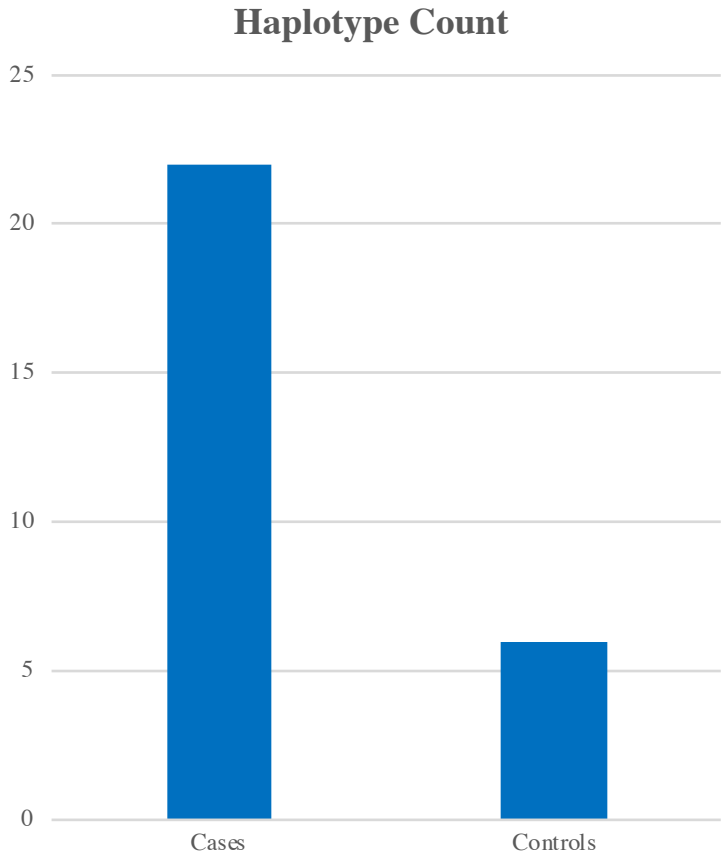


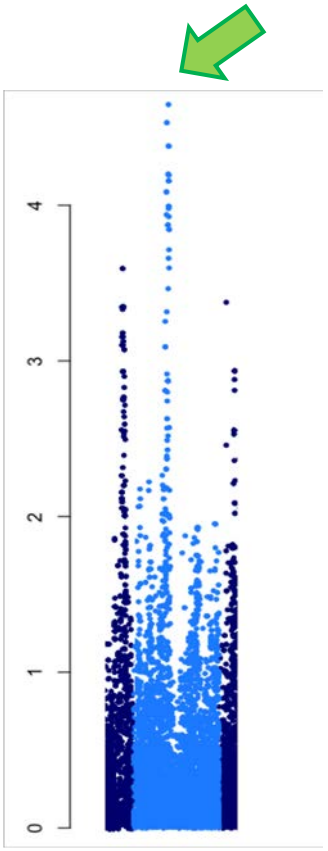
Current dataset

Batch 6



Right Population





Total Population

What has been found and what will be done

• Cataracts in the ACS is more complicated than originally suspected.
• It is not a single gene (i.e. monogenic) disorder (otherwise the Manhattan plot would have given a single sharp peak), but cataracts are caused by at least two different genes that result in cataracts that are clinically indistinguishable. There is likely to be a 3rd gene that is a disease modifier.
• Although WGS efforts will focus on dogs in the >2 - 5< yr range (to simplify our effort), GWAS does not distinguish between these dogs and those that are older (>5 – 8<) or with those that have symmetrical bilateral cataracts or unilateral cataracts that over the span of several years develop cataracts in the second eye.
• **Now that we have zeroed in on the chromosomal regions of interest, WGS is being done in 4 controls and 4 cases that have the haplotypes for the chromosomes being studied.** More will be done on an as needed basis.