Stargardt disease in Labrador Retrievers

Dr. Katy M. Evans, The Jane H. Booker Chair in Canine Genetics

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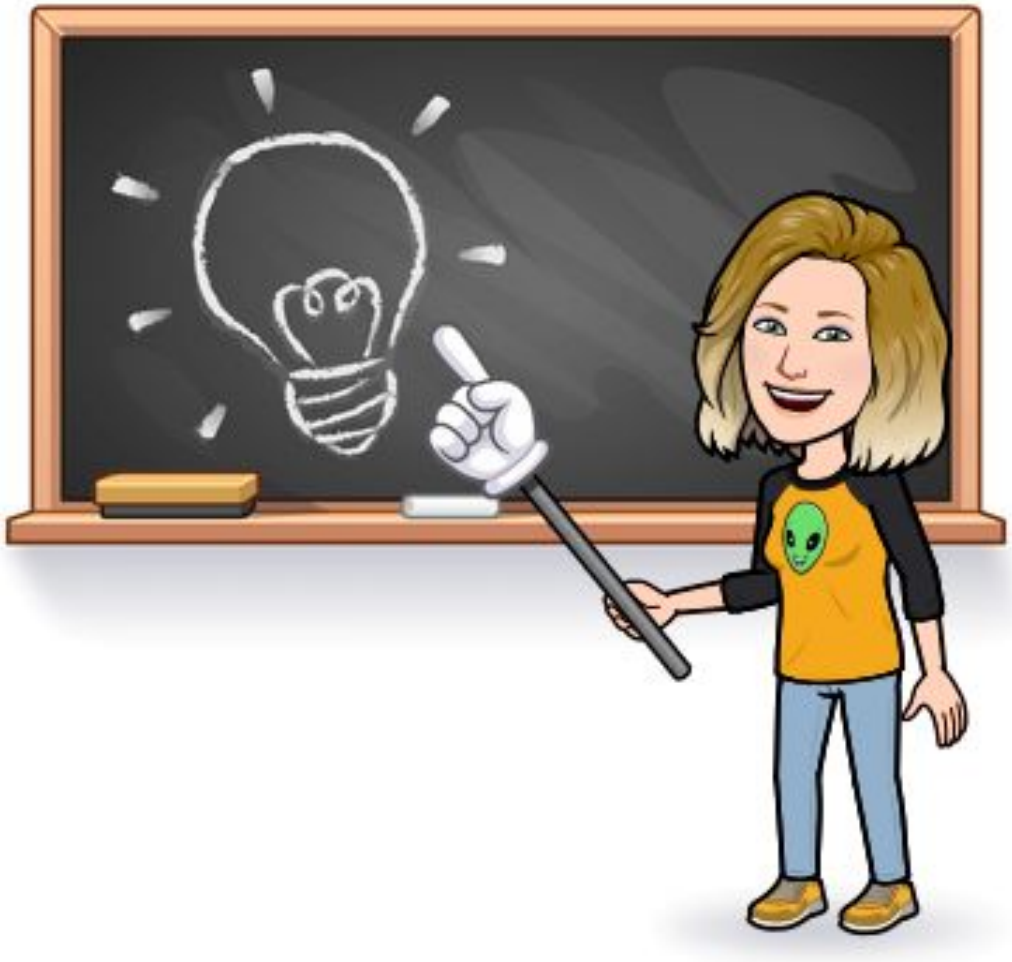
Independence & Dignity Since 1929



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DNA Genetic Code Dictates Amino Acid Identity and Order

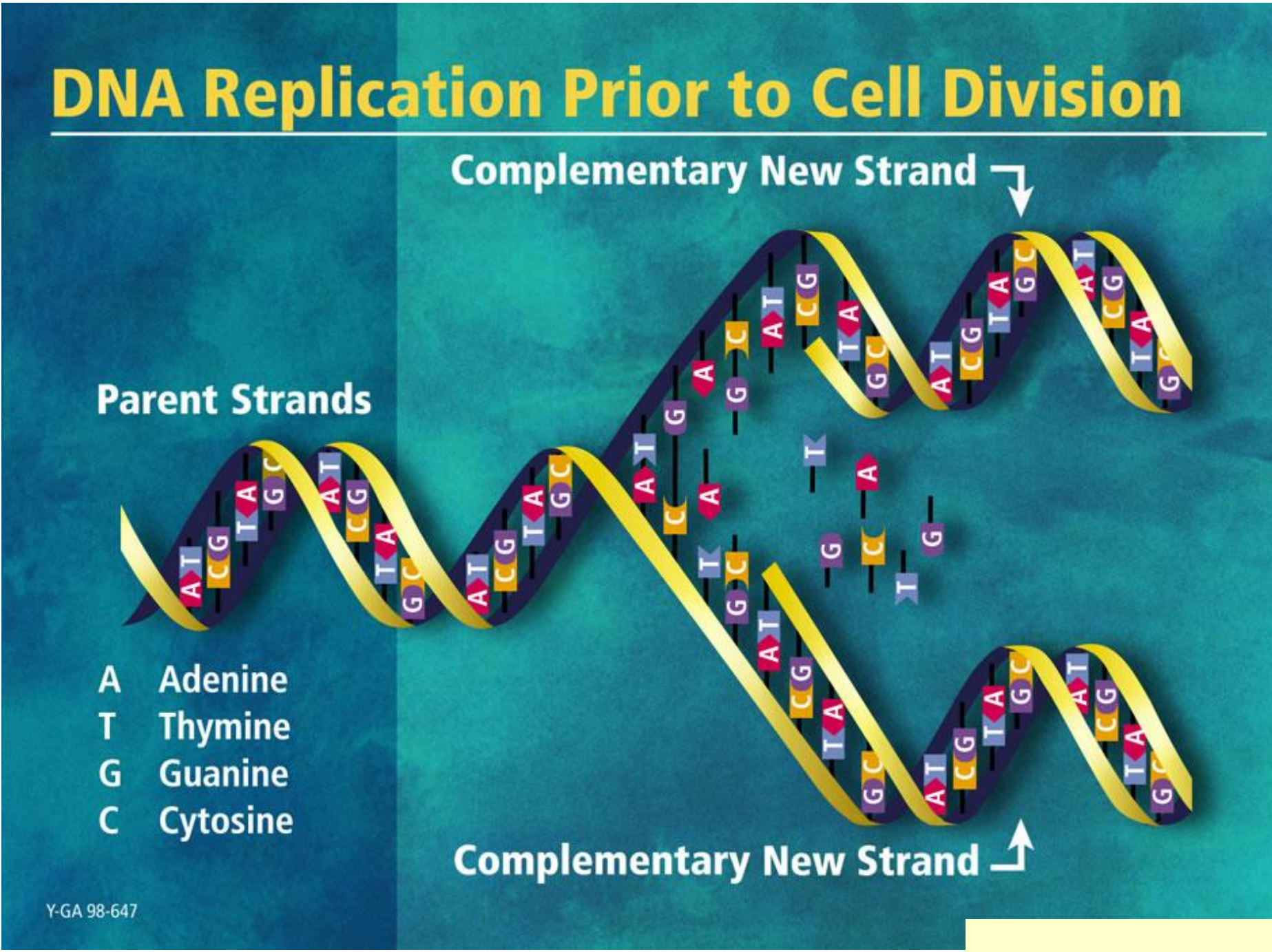
DNA Sequence Is the Genetic Code.

GCA AGA GAT AAT TGT...

. . . Growing Protein Chain

Y-GA 98-648

DNA Replication Prior to Cell Division

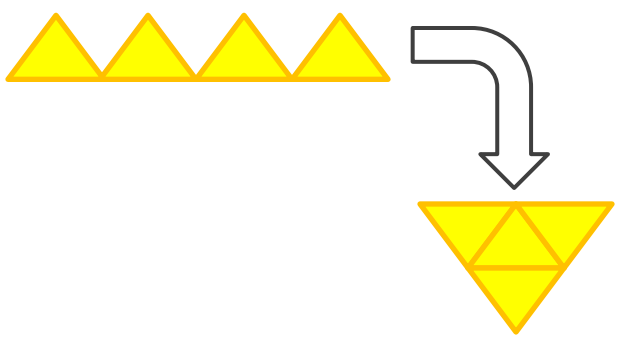


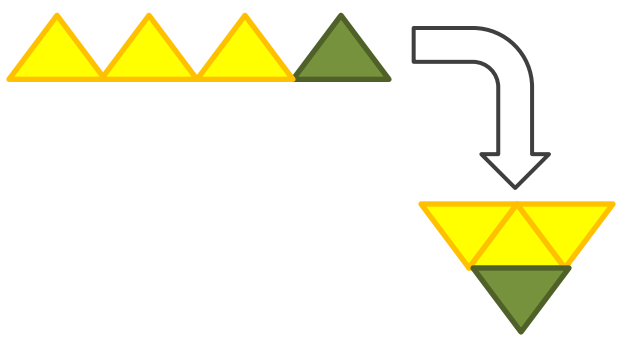
Slide from Dr. Tom Lewis

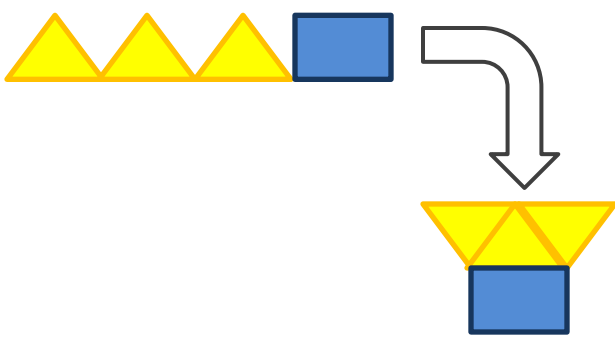
CGT-CGT-CGT-CGT-TGA

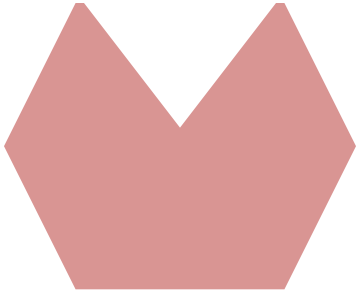
CGT-CGT-CGT-CGC-TGA

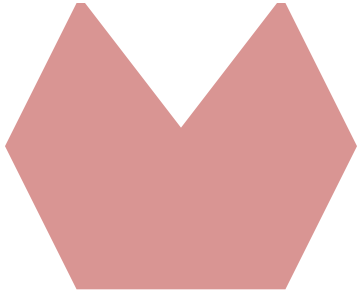
CGT-CGT-CGT-CAT-TGA

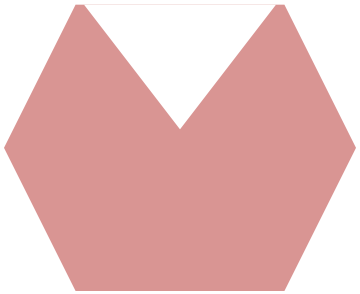






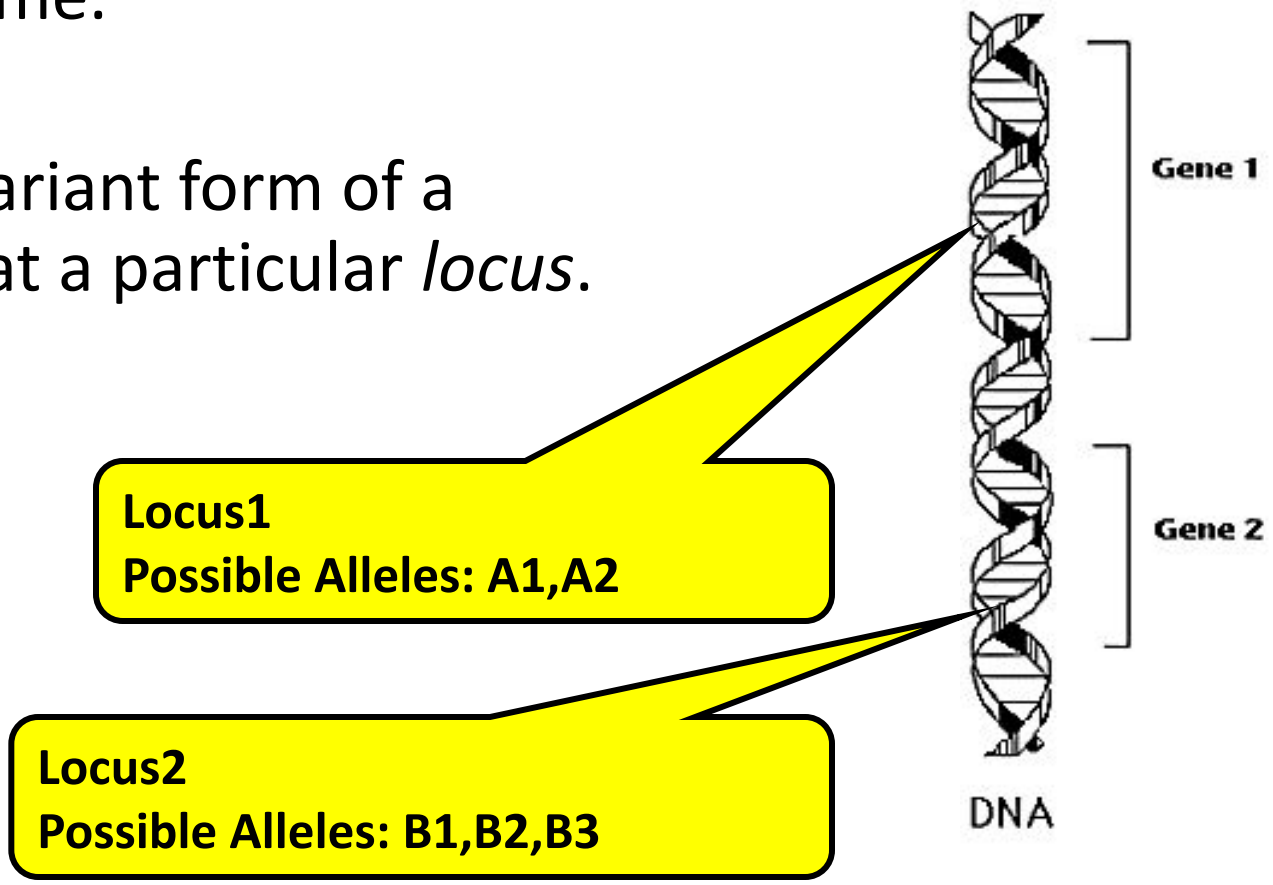






Chromosome Logical Structure

- **Locus** – location of a *gene/marker* on the chromosome.
- **Allele** – one variant form of a gene/marker at a particular *locus*.



Genotypes  Phenotypes

- At each locus (except for sex chromosomes) an individual has 2 alleles. These constitute the individual's ***genotype*** at the locus.

- The expression of a genotype is termed a ***phenotype***. For example, coat color, height, or the presence or absence of a disease.

Dominant vs. Recessive

In Labradors the brown (B/b) locus controls the expression of the dark pigment eumelanin.

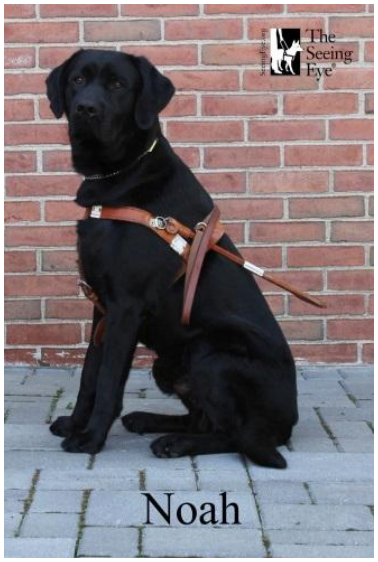
A ***dominant*** allele is expressed even if it is paired with a recessive allele e.g. in Labradors B = black

A ***recessive*** allele is only visible when paired with another recessive allele e.g in Labradors b = brown

When an individual has two copies of the same allele at a locus, e.g. BB or bb, they are described as **homozygous**

An individual with two different alleles at a locus, e.g. Bb, they are described as **heterozygous**

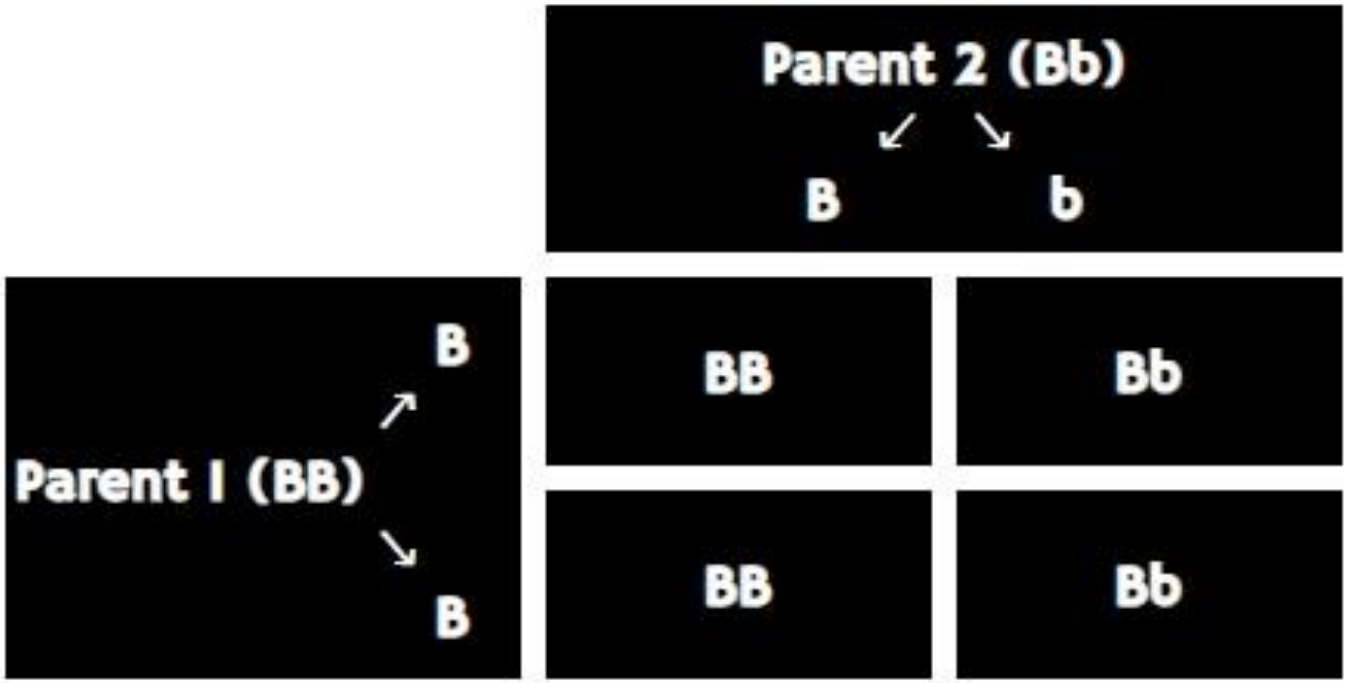
The phenotype is clearly visible, but the genotype is only visible if the individual is **homozygous recessive**



BB or Bb

bb

One gene = ‘simple inheritance’



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Stargardt disease

- Seven patients with a recessively inherited macular dystrophy were described by Karl Bruno Stargardt at the University of Marburg, Germany, in 1909
- The most prevalent inherited form of macular dystrophy
- Affects approximately 1 in 10,000 people

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Stargardt disease 2

- The majority of cases are caused by recessive mutations in *ABCA4* gene, first identified in 1997

- Vision loss typically starts before 20 years of age

- Common symptoms:
  - gradual loss of central vision in both eyes
  - light sensitivity
  - needing more time to adjust between light and dark places

Aetiology

- The *ABCA4* gene normally encodes a protein that transports byproducts away from the photoreceptor (rods and cones) cells in the retina
- Failure to remove the byproducts results in accumulation of lipofuscin, which is highly toxic to the retinal pigment epithelial (RPE) cells
- The rods and cones rely on metabolic support of the RPE cells, so the gradual destruction of the RPE cells results in gradual loss of rods and cones

Aetiology 2

- Faster rate of progression associated with:
  - increased sunlight/UV light exposure
    - climate
    - altitude
    - lifestyle
  - high vitamin A intake
  - potentially other, unidentified, genetic factors

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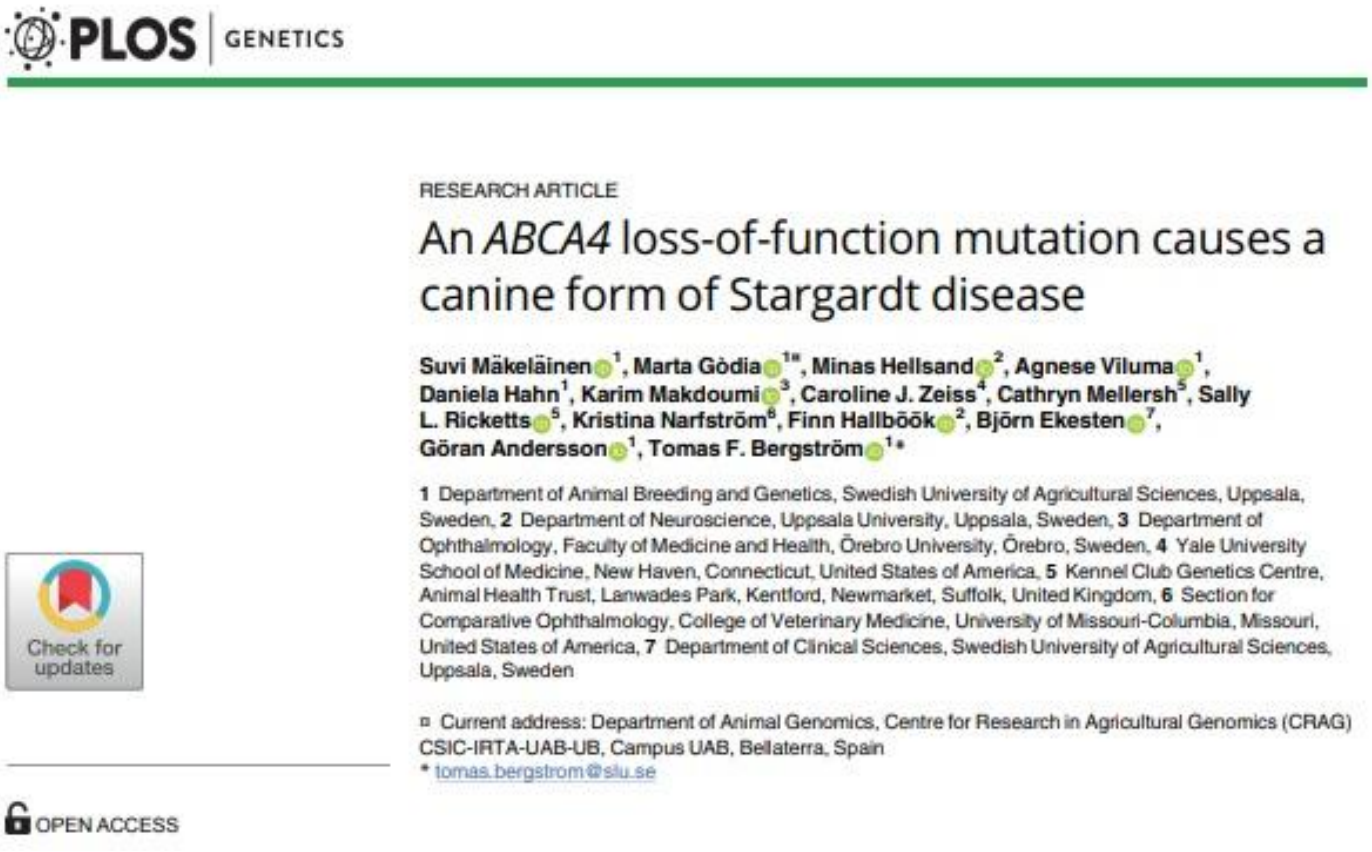
Stargardt disease in Labradors

- Paper published 2019



PLOS | GENETICS

RESEARCH ARTICLE

An *ABCA4* loss-of-function mutation causes a canine form of Stargardt disease

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Stargardt disease in Labradors 2

- Vision of affected dogs described as impaired in both daylight and dim light conditions by 10-12 years of age
- Some vision retained throughout their lifetime
- Ophthalmoscopic signs of bilateral diffuse retinal degeneration
- Reduced number of photoreceptors; cone function profoundly abnormal, rod function better preserved (ERG abnormal)

Stargardt disease in Labradors 3

- As with the majority of human cases, caused by a recessive mutation in the *ABCA4* gene (on CFA 6)
- Whole genome sequencing (WGS) of a family quartet (sire, dam and two affected offspring)
- WGS findings validated in 16 related and 6 unrelated Labradors
- DNA test made commercially available

Stargardt disease investigation at The Seeing Eye

- Mutation added to our DNA panel test in September 2020
- At that time 5 of our 29 Labrador (or backcross) breeders were carriers
- Decision made to avoid mating carriers together

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Stargardt disease investigation at The Seeing Eye 2

- Our expert veterinary ophthalmology consultant, Dr. Gus Aguirre, advised that homozygous-affected dogs should not work as guide dogs
- Went back through pedigrees behind our known carriers to identify older carrier (or affected) breeders
- Aim to identify status of all parents with offspring still in our program

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The Seeing Eye's 'biobank'

- Since 1999, almost every dog born has had blood and/or DNA stored
- Since 2016, all puppies' dew claws are also stored (including stillborns)

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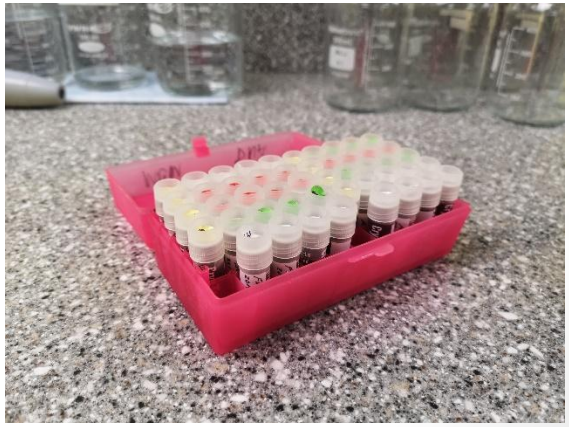
The Seeing Eye's 'biobank' 2

- Blood is collected into citrate anticoagulant
- Three 1.5ml vials of blood are frozen (at -20°F)
- The rest used for DNA extraction; extracted DNA is then also frozen, split into multiple tubes
- Good success with using frozen blood or DNA for SNP genotyping and WGS



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Stargardt disease investigation at The Seeing Eye 3

- Used stored DNA for genotyping of all parents with offspring in our program

- Where carrier (or affected) to carrier mating had occurred, used stored DNA to genotype all dogs in the litter

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Our situation

- Last litter (of 7) from two carrier parents born February 2020 – tested when they came in for training, 5 carriers, 1 clear, 1 affected
- Two dogs in training identified as affected (two carrier siblings, qualified and working)
- Eight working guide dogs (including one former breeder) identified as genetically affected

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Youngest affected dog

- No fundic abnormalities found on ophthalmic examination by expert at 1y9m & 2y10m

- Normal ERG, showing normally functioning cones and rods, at 1y9m (repeat scheduled)

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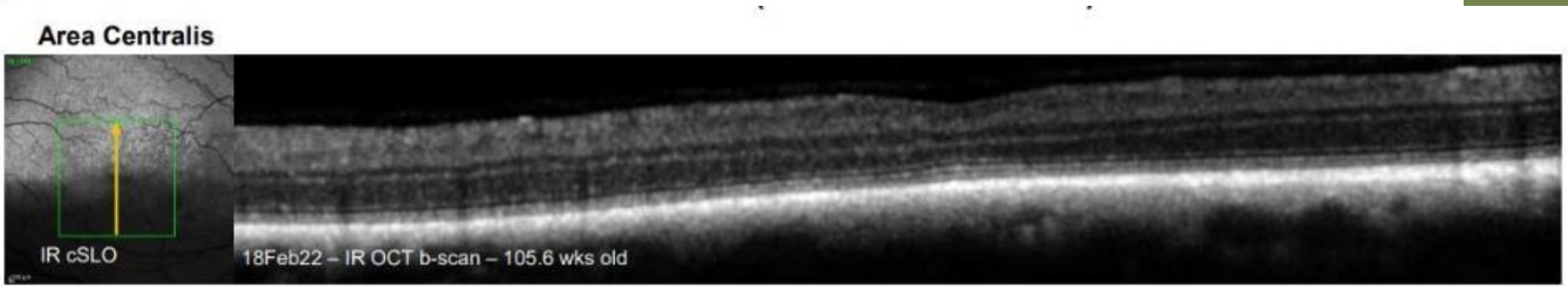
Youngest affected dog's OCT results (at 2y)

- Generally normal fundus
- Marked thinning of the retina and outer nuclear layer at the area centralis (foveal dip)



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Two brothers in training

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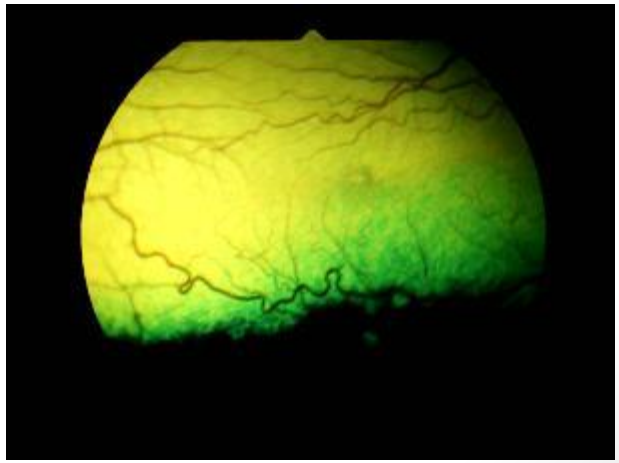
Dog 1 in training

- Fundic abnormalities found on ophthalmic examination by expert at 2y4m – focal changes of reflectivity in area centralis, symmetrical both eyes

- ERG at 2y3m – slight reduction in scotopic responses compared to control dogs, marked reduction in photopic responses



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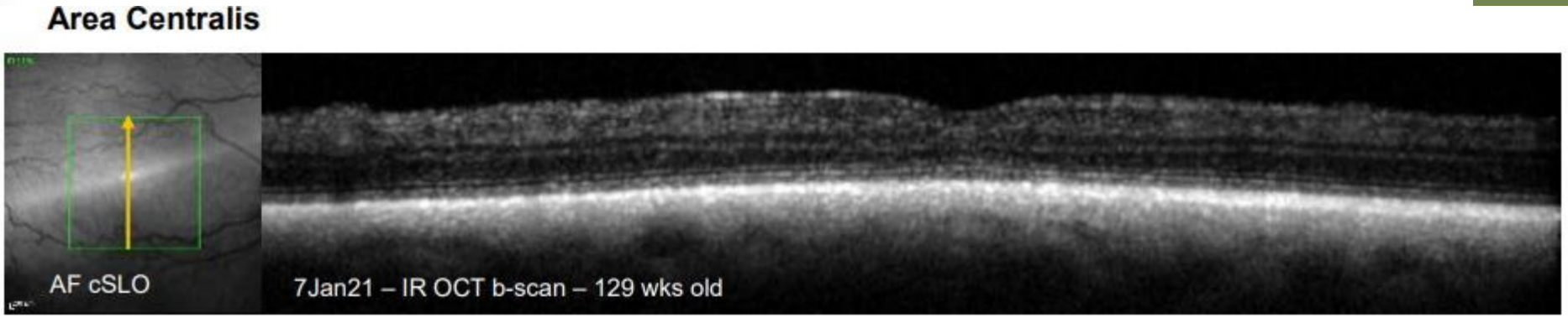
Dog 1 in training

- OCT at 2y6m – marked thinning of the outer nuclear layer at the area centralis, corresponding to the area of greatest intensity on the autofluorescence confocal scanning laser ophthalmoscopy



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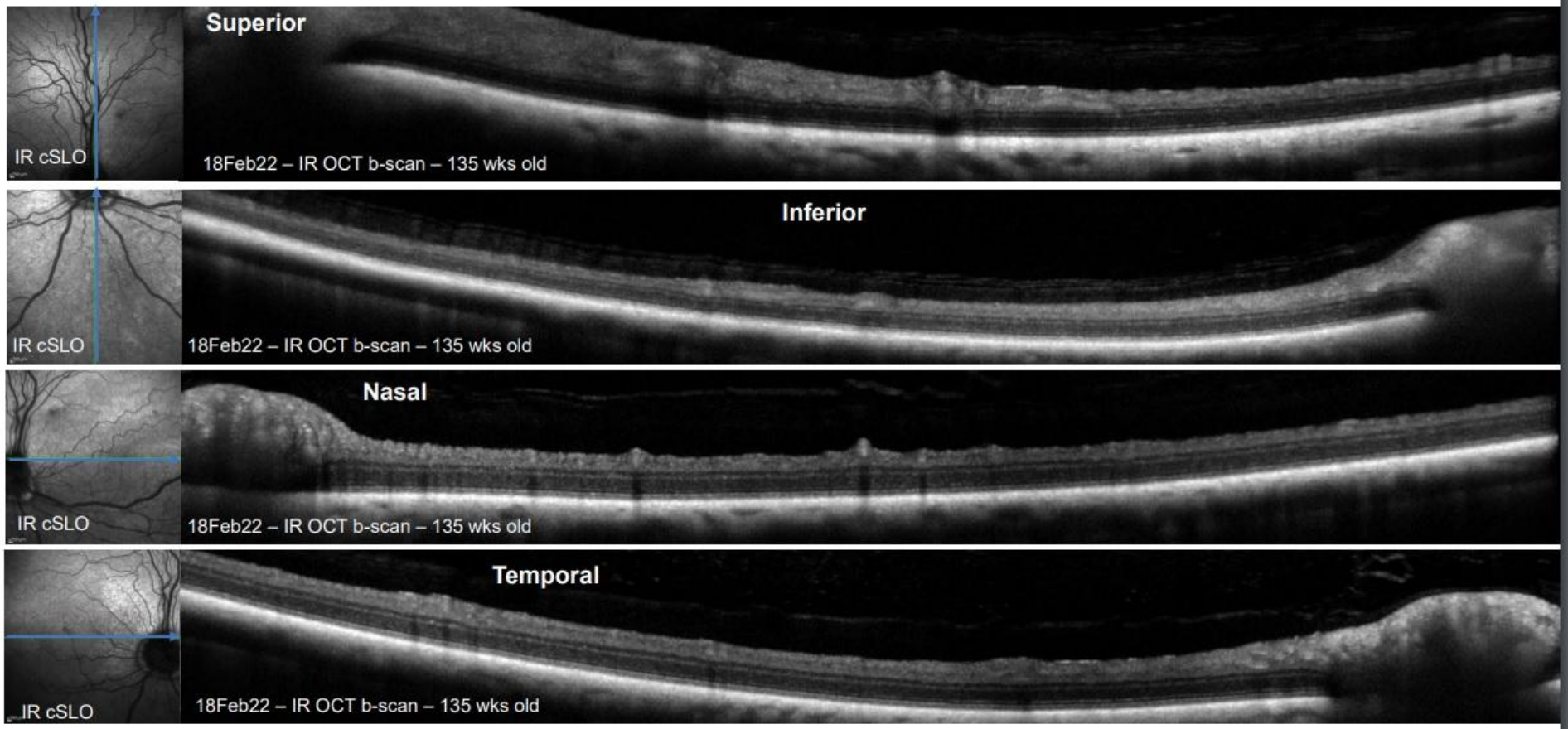
Dog 2 in training

- No fundic abnormalities found on ophthalmic examination by expert at 2y4m – area centralis normal in both eyes
- Repeat expert fundic exam at 3y8m – still normal
- ERG at 2y4m – moderate reduction in scotopic responses compared to control dogs, marked reduction in photopic responses

Dog 2 in training

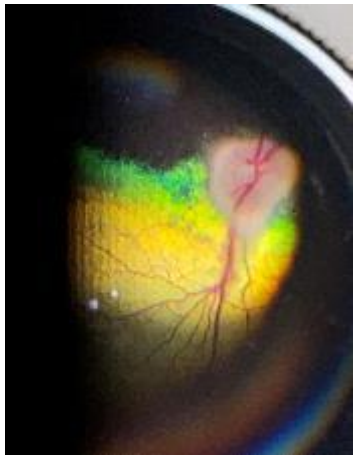
- OCT at 2y7m – a generally normal fundus with some slight thinning of the ONL at the AC (within normal limits) and an area of greatest intensity on AF cSLO

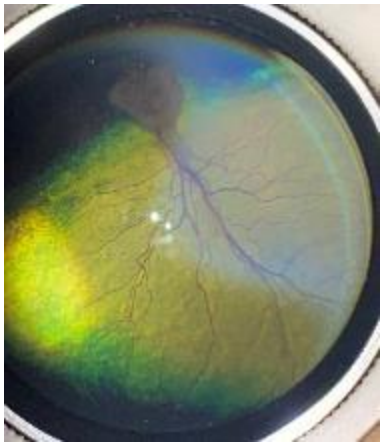


Youngest guide dog

- Fundic abnormalities found on ophthalmic examination by expert at 7y4m – focal hyperreflective spot at area centralis, generalized foci with change in reflectivity

- ERG at 7y4m – normal scotopic waveforms although possibly reduced in amplitude, minimal to no photopic responses bilaterally





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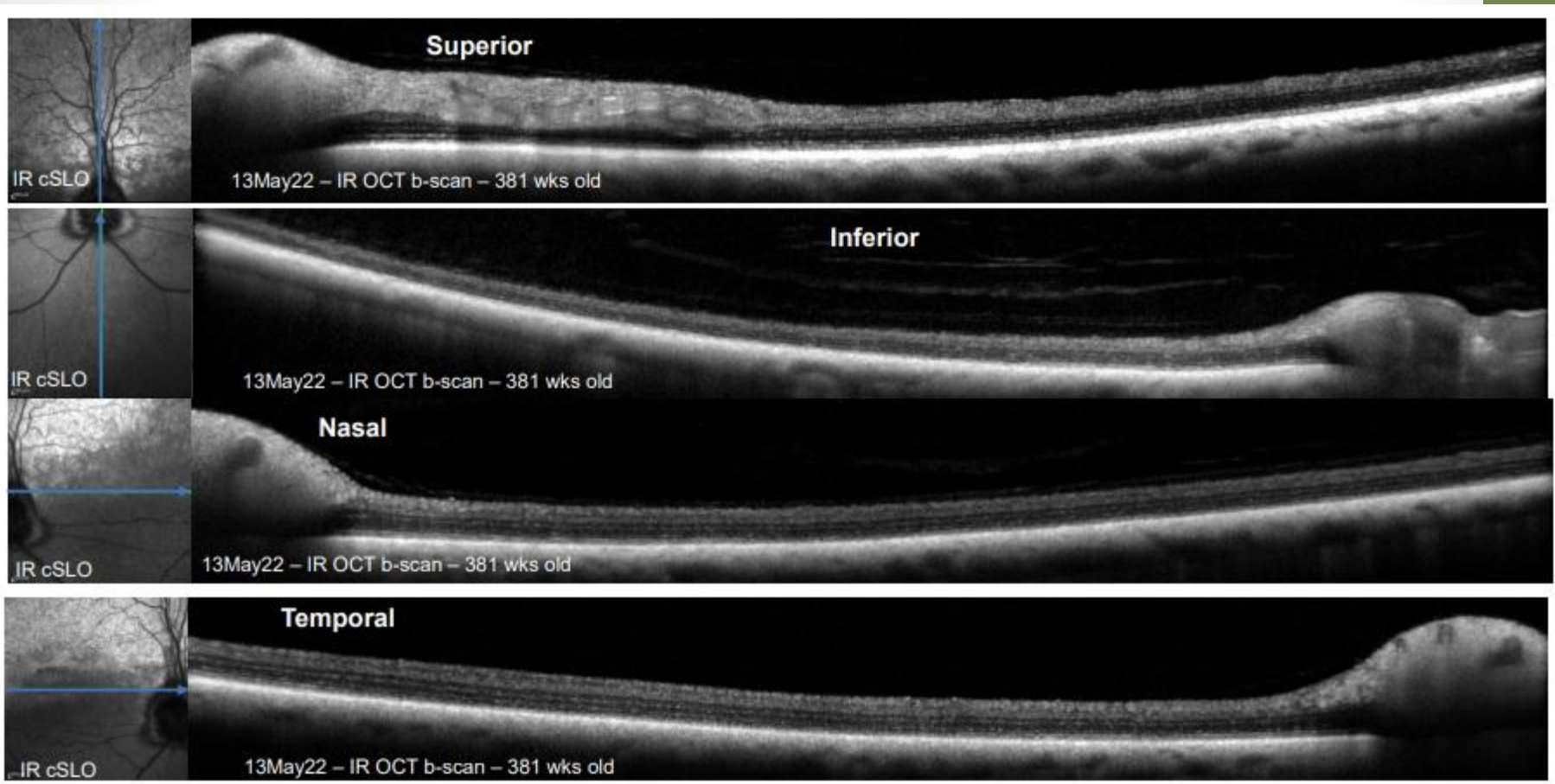
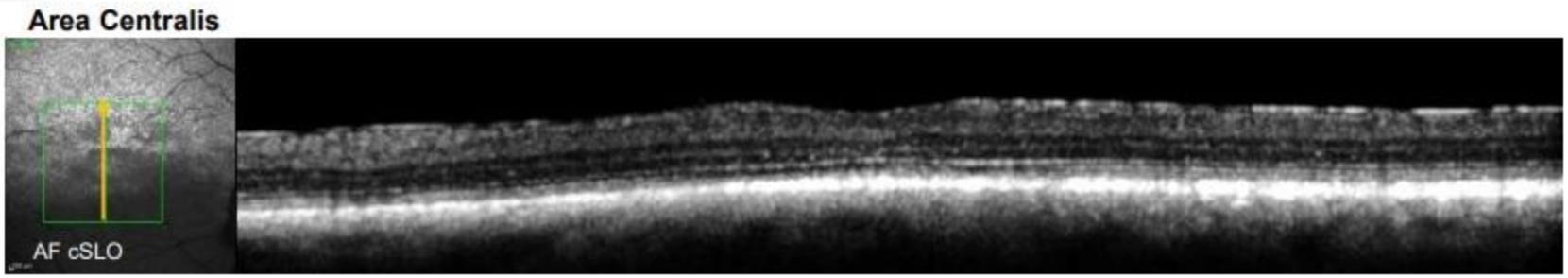
Youngest guide dog





Youngest guide dog

"She did not work like a typical seasoned dog. Despite the fact that they are with a new handler (me), most seasoned dogs move out with confidence. She seemed to lack confidence and was most affected going from light into darkness. She was hesitant to approach down curbs and storm drains, yet would approach ramps. She avoided manhole covers as if they were open holes in the sidewalk, yet walked over all gratings as if they were not there."

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Guide dog 2

- No fundic abnormalities found on ophthalmic examination by expert at 8y1m
- ERG at 8y3m – very abnormal cone responses, either absent or very low amplitude at all frequencies
- OCT not performed

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Guide dog 2

"When I worked her facing into bright sunlight her pull diminished. In fact, I could not get her to accept the harness facing into the sunlight, but when I turned to harness her with our backs to the sun she accepted the harness readily. When working her in the dark she did not like working into oncoming car headlights – her pull diminished with uncertainty. When working her into a dark driveway with heavy shadow from overhead trees she was reluctant to work at all. I should note she seemed perfectly happy in daylight and cloud cover as long as the sun was not shining brightly in her eyes"

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Guide dog 3

- GDO could not bring the dog to campus for investigations and instead requested a visit with a veterinary ophthalmologist in her area

- Report at 8y1m: ‘Owner says dog has had issues in dim light and seems most comfortable when the light is better. Owner indicates that dog is not guiding her across streets in the dark at this point. Suspect that even though fundi appear normal, dog could have some rod function issues.’

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Guide dog 4

- Fundic abnormalities found on ophthalmic examination by expert at 8y10m – focal hyperreflective spot over AC
- ERG at 8y10m – normal scotopic waveforms although possibly reduced in amplitude, minimal to no photopic responses
- OCT at 9y – thinning of ONL in all 4 quadrants, markedly thinned ONL at AC

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Guide dog 4

"My route began with traveling from east to west with sunlight overhead, broken up by buildings on our right. She still moves out like a young, spry dog. She trotted along happily at a brisk pace with a defined pull all the way to the end of the block. When we turned north, into the shade, there was some initial diminished pace and pull for 50-100 feet. I supposed that she was adjusting to the diminished light. What was very clear was her reaction to bright spots of sun shining on the pavement. She made very strong, overt moves to guide me around those spots without breaking stride, as if they were unsafe obstacles…"

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Guide dog 4

"… At the end of the shady block we wound up in bright sunlight and turned to the east. It's interesting to me that there doesn't seem to be as much of an adjustment walking into bright sunlight as there is walking into darkness and shadows – for this dog and the others. On the next dark, shaded block, traveling south, I once again observed a more tentative dog until the conditions became brighter again."

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Guide dog 5

- Fundic abnormalities found on ophthalmic examination by expert at 10y10m – peripheral naso-temporal tapetal multifocal mottling with hyperreflectivity, in both eyes

- ERG at 10y10m – abnormal rod responses, very low amplitudes at all intensities and frequencies; cone responses absent

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Guide dog 5

“She seemed vibrant in her work during normal daylight with grad and with instructor who worked her into the sun. When I worked her at night, into oncoming headlights on a dark road, her pull decreased and she seemed uncertain. Her reaction to darkness was marked. She froze in dark shadows and was uncertain”

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Guide dog 6

- Fundic abnormalities found on ophthalmic examination by expert at 10y10m – mottling of fundus and focal areas of tapetal pigmentation bilaterally

- ERG at 10y10m – normal rod, absent cone, responses

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Latest research

- Paper published 2022

tvst

Article

Abnormal Appearance of the Area Centralis in Labrador Retrievers With an *ABCA4* Loss-of-function Mutation

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Correspondence: Björn Ekesten, Department of Clinical Sciences,

Purpose: To study retinal appearance and morphology in Labrador retrievers (LRs) heterozygous and homozygous for an *ABCA4* loss-of-function mutation.

Latest research findings

- Studied 6 homozygous affected, 5 heterozygous (carriers) and 5 homozygous wildtype (clear)
- Clinical findings in homozygous affected dogs same as ours
- Clear evidence of visual impairment in both daylight and dim light in dogs >8 years of age
- Ophthalmoscopic examination and vision testing of carriers normal at all ages

Selection strategy

- Homozygous affected and carrier dogs can safely be used as breeders, **so long as mates are clear**
- Try to reduce mutant allele frequency slowly so as not to reduce genetic diversity
- Could preferentially choose clear progeny over carrier littermates

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Summary

- Fundic abnormalities found on ophthalmic examination by expert at 2y4m – but relatively non-specific
- OCT abnormalities found at 2 years
- ERG abnormalities detected at 2y3m
- Work abnormalities detected, although not by GDO, at 7y4m (youngest dog checked)

Summary

- Our strong opinion is that it is unsafe to place a genetically affected (homozygous) dog as a guide

- Genetically affected dogs can safely be used as breeders so long as they are only mated with clear dogs

- Carriers safe for work and breeding

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Acknowledgements

- Dr. Dolores Holle
- Dr. Kyle Quigley
- David Johnson
- Patricia Tardive




- Dr. Gustavo Aguirre

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