The Cerebellar Saga:

Why do Dogs with CA Wobble?

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The Problem

* Neurodegenerative disease that produces progressive gait abnormality

First known case reported in the UK in 1979

* Larger cluster of cases appeared in the US in the 1990s

Cerebellar degeneration in Old English Sheepdogs

H. Steven Steinberg, VMD, DACVIM; Thomas Van Winkle, VMD, DACVP; Jerold S. Bell, DVM; Alexander de Lahunta, DVM, PhD, DACVIM

Gait abnormality

* Onset of signs between 6 months and 3.5 years

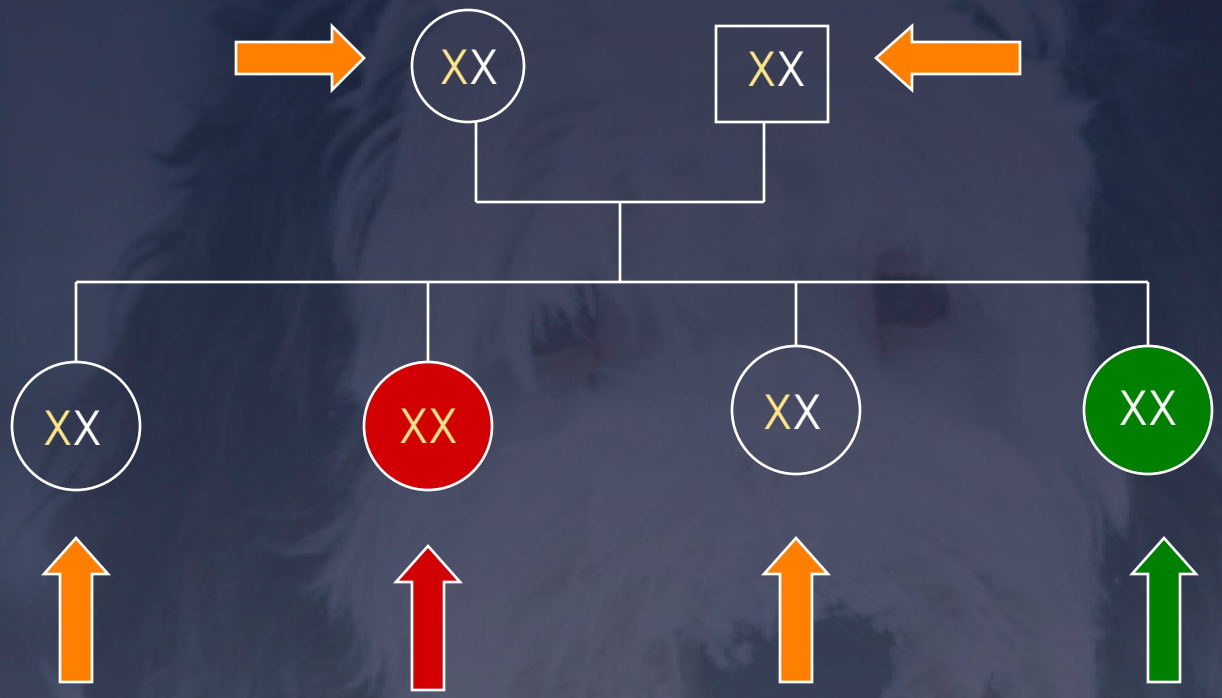
Truncal Sway

Intention Tremor

Difficulty with Stairs

Mode of Inheritance

- Autosomal recessive
- Need 2 copies of the abnormal gene to show signs
- Dogs with one copy are silent carriers that can pass the mutation on to their offspring but never show signs

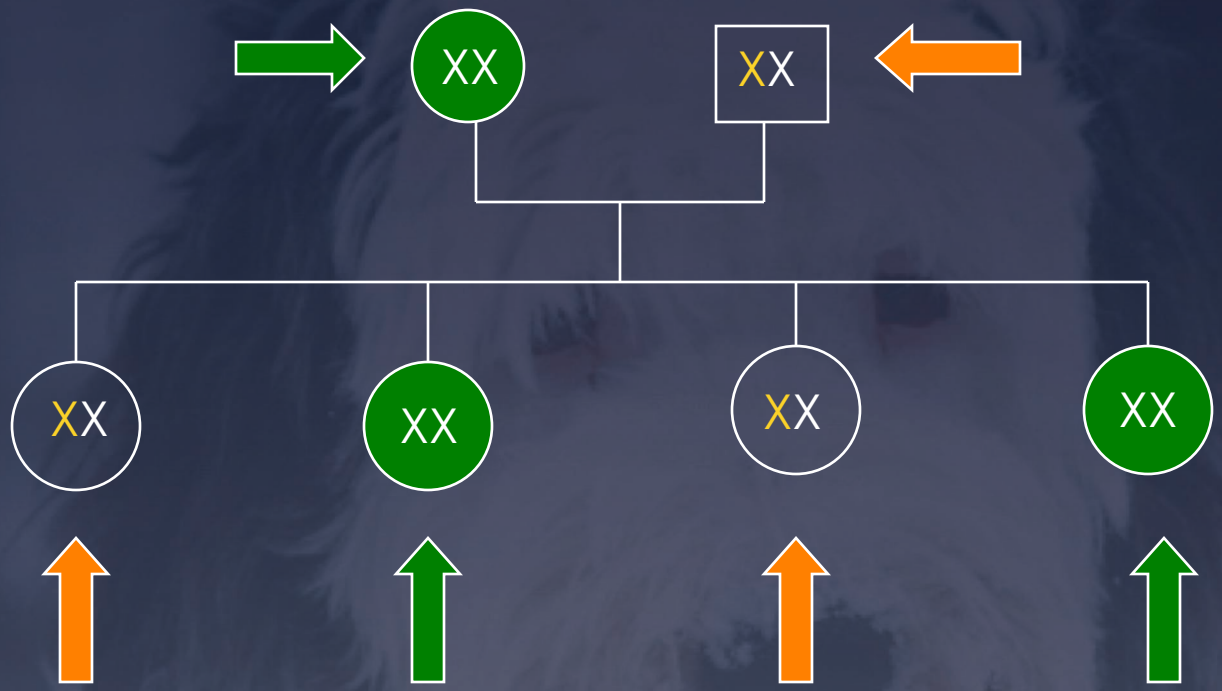


Affected

Normal carrier

Normal

X: abnormal (mutant) gene
X: normal gene



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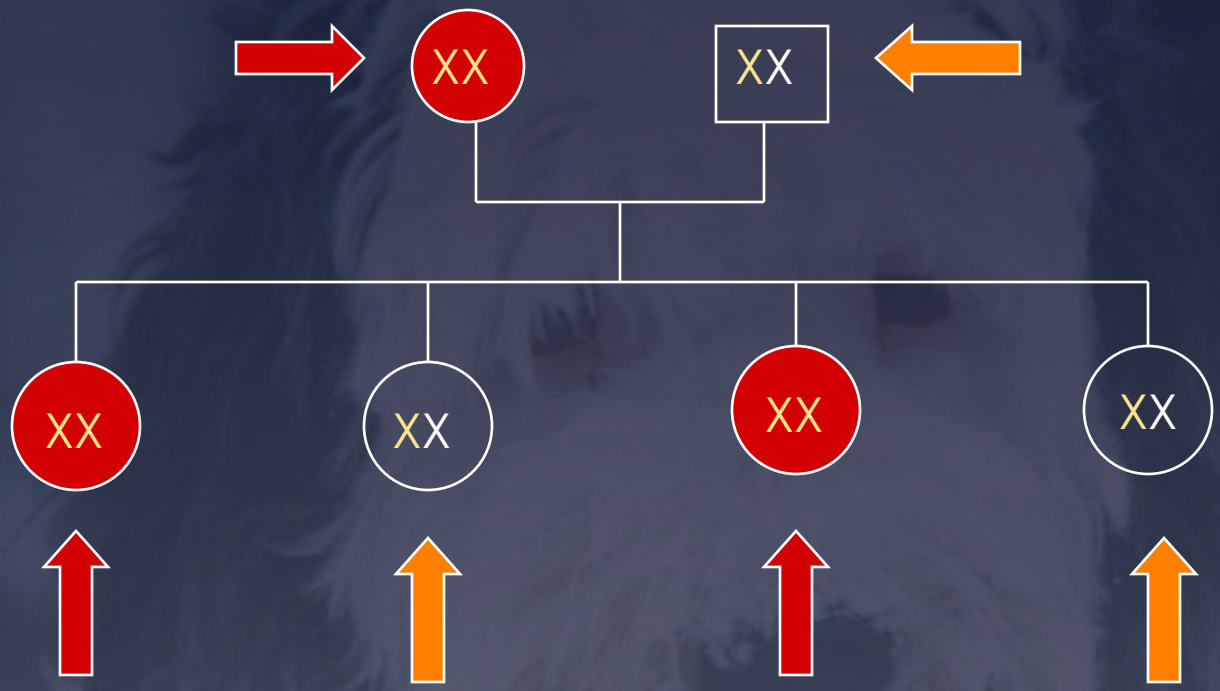

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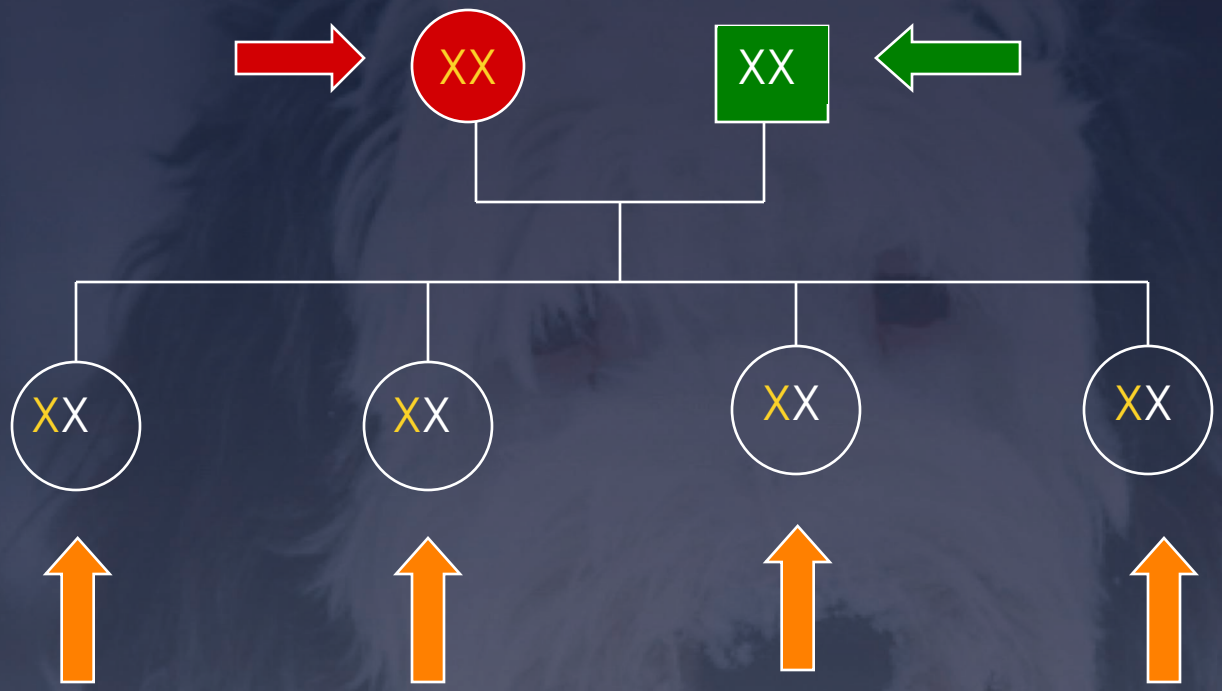

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Normal

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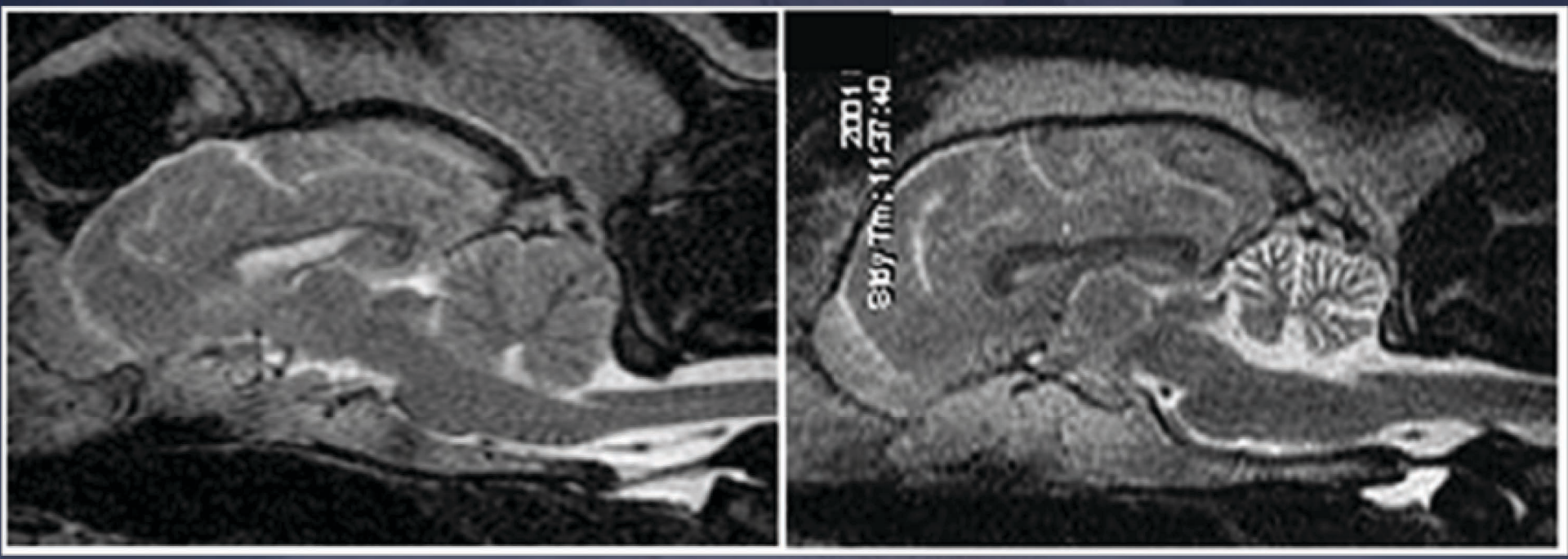
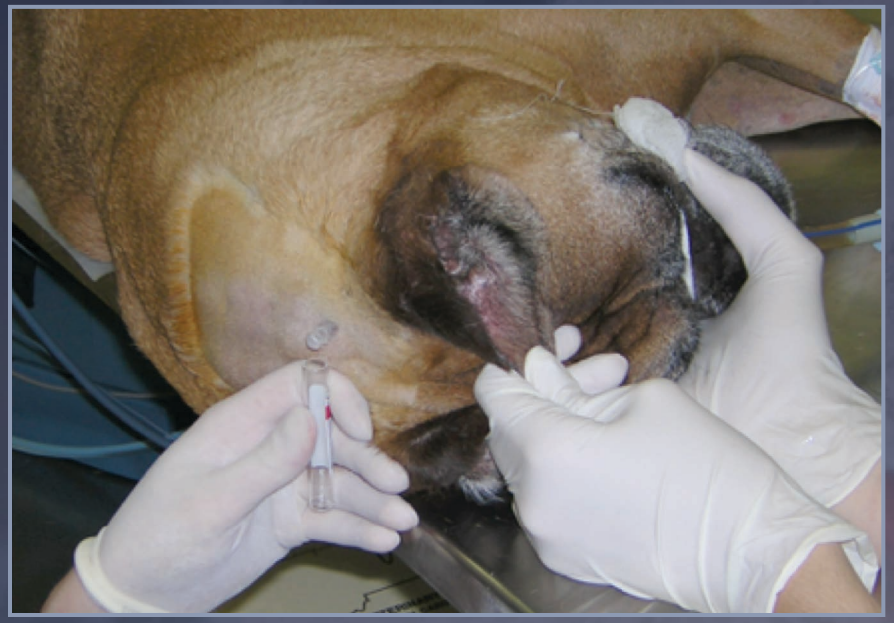
Diagnostic Test Findings: MRI

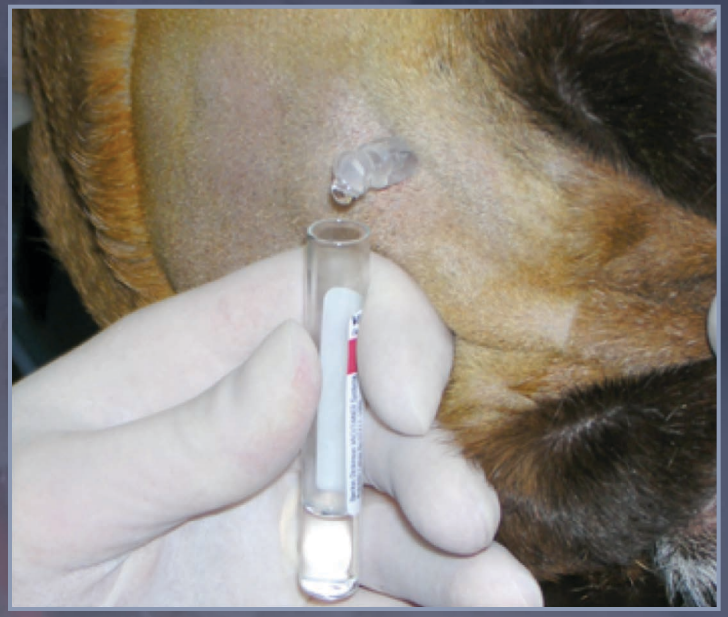


Thames et al., 2010

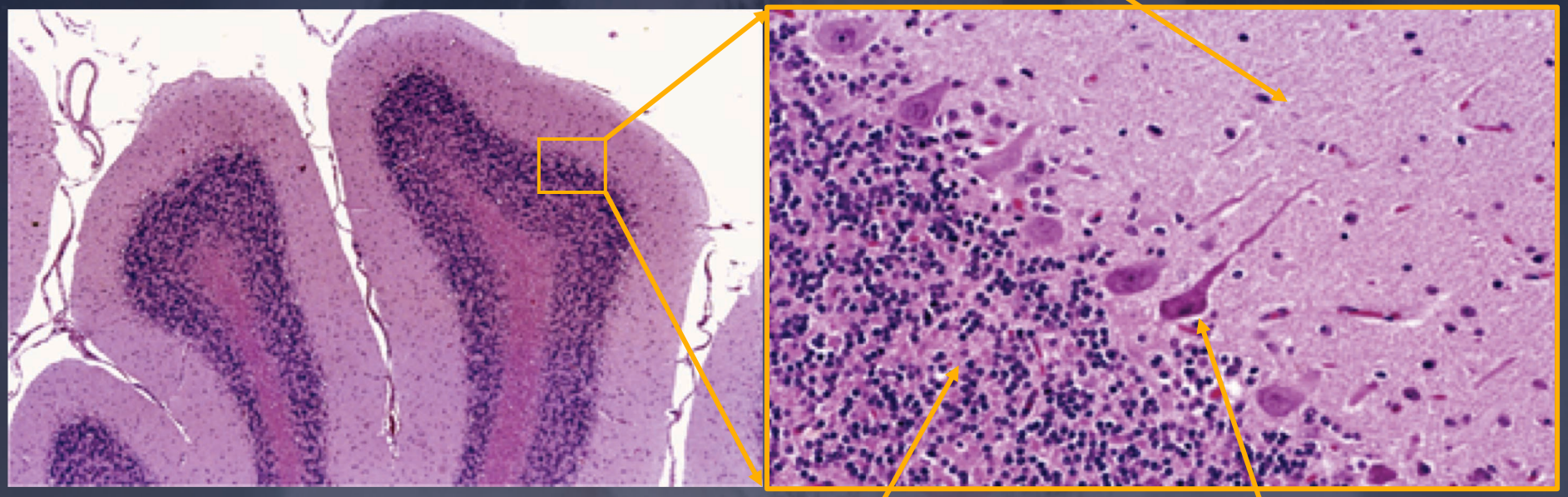
CSF analysis - spinal tap

- *Will allow the clinician to diagnose encephalitis*
- *Performed under general anesthesia*
- *Normal – no evidence of inflammation*

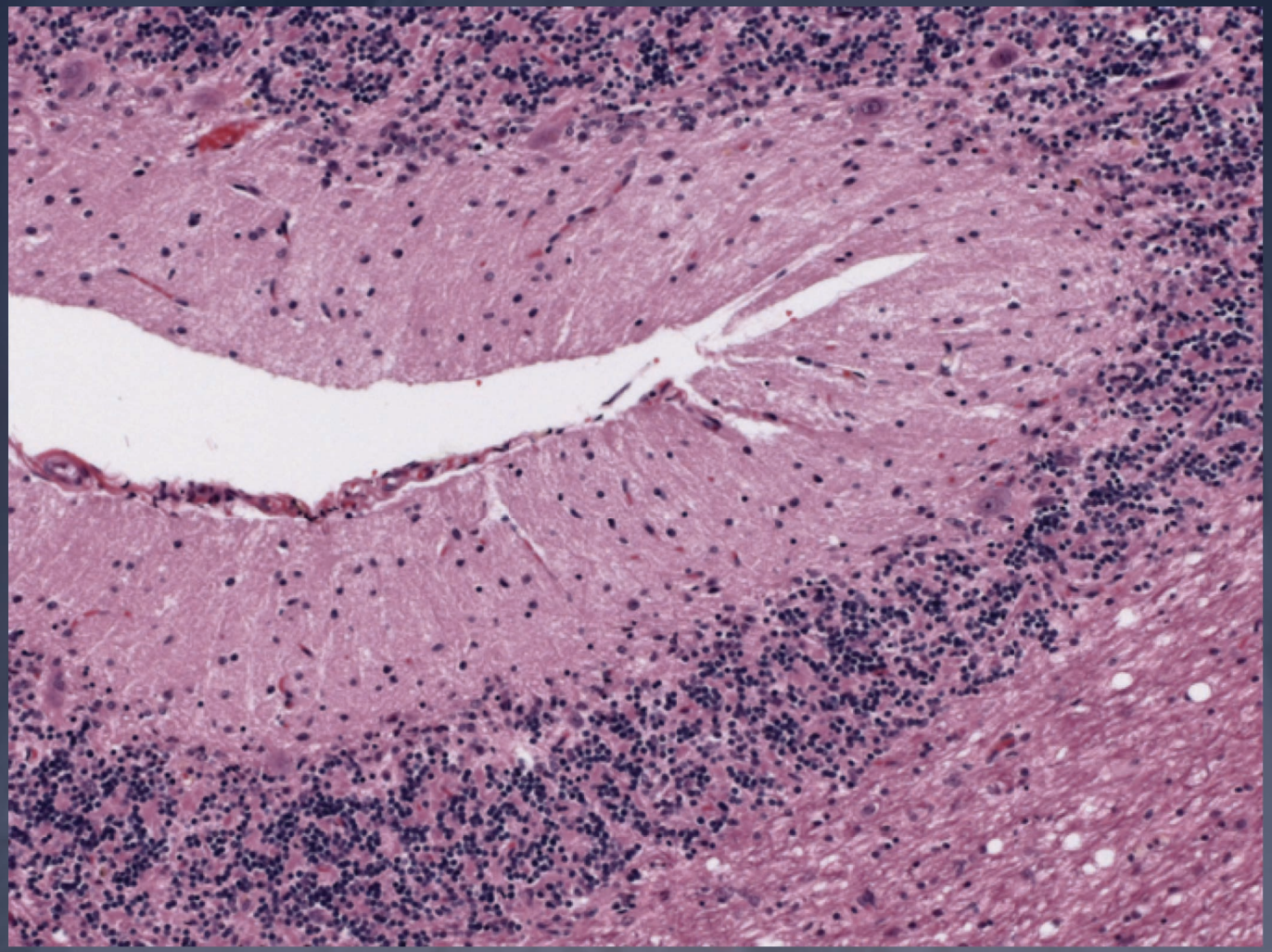




Histopathology: normal CBM

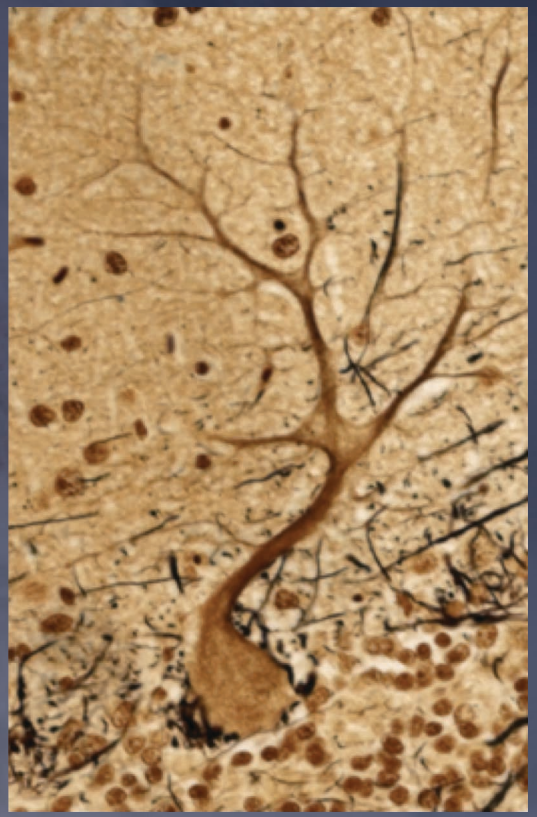


Histopathology: Affected Dog



The Cerebellum

- Controls the rate, range and force of movement
- Vital for smooth, coordinated movement



Neurodegenerative Diseases of the Cerebellum: Humans

* Hereditary Ataxias or Spinocerebellar Ataxias (SCA)
* Autosomal recessive, dominant, X linked or mitochondrial
* Onset at a wide variety of ages
* Often also affect another region of the nervous system
* Numerous different genetic causes known, but the cause in many people remains obscure

Neurodegenerative Diseases of the Cerebellum: Dogs

* Cerebellar Abiotrophy, Cerebellar Ataxia, Cerebellar Cortical Degeneration, Cerebellar Degeneration
* Autosomal recessive or X linked
* Onset at a wide variety of ages
* Genetic cause known in 2 breeds – the beagle and the Finnish Hound

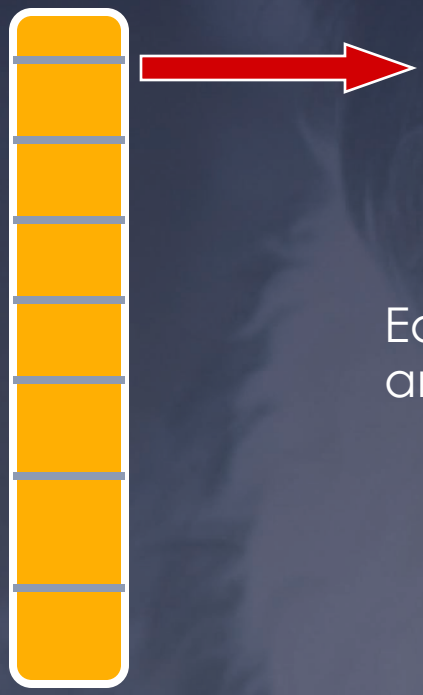
The Start of the Search: 1990s

- DNA samples were collected on a large number of Old English Sheepdogs (*Jerry Bell et al.*)
- Phenotype was recorded and method of phenotype determination was noted

- A candidate gene approach was used to try to find the mutation
- 5 genes were sequenced and none were abnormal(*Gary Johnson et al)*

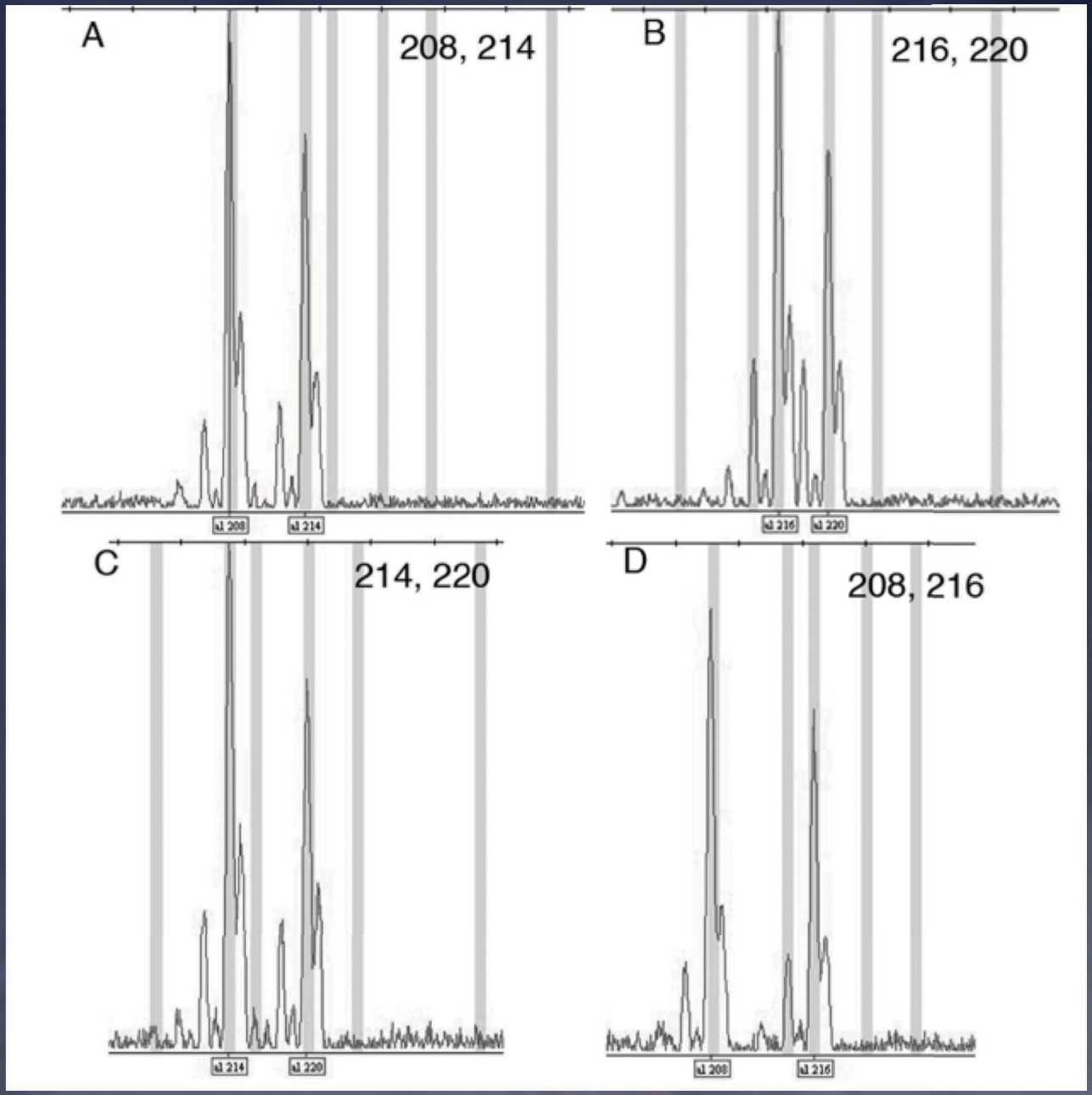
Step 2: Linkage Analysis (2006-2008)

Process in which the *phenotype* is linked to the *genotype* specific markers positioned across all of the chromosomes



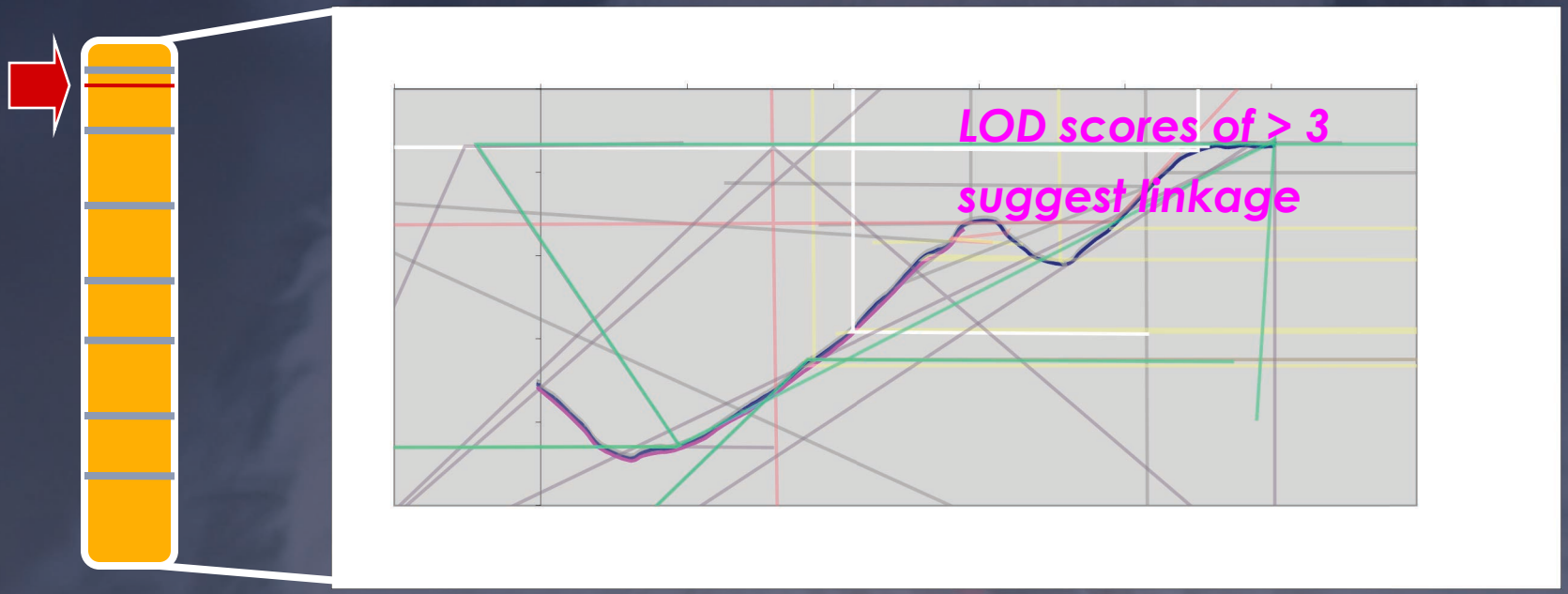
Each ‘marker’ has been selected because there are several different versions - alleles

Each allele will be passed from parents to offspring and can be traced down the generations


A
208, 214
B
216, 220
C
214, 220
D
208, 216

Linkage Analysis contd

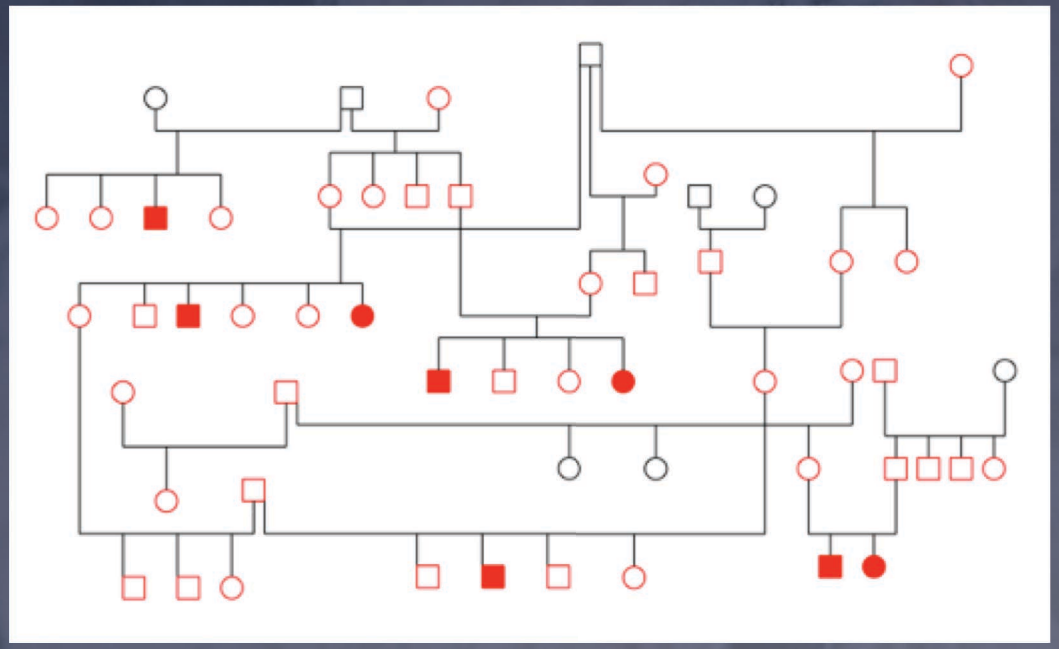
It is possible to link the inheritance of phenotype (affected or normal) to the inheritance of certain genotypes….*linkage analysis*



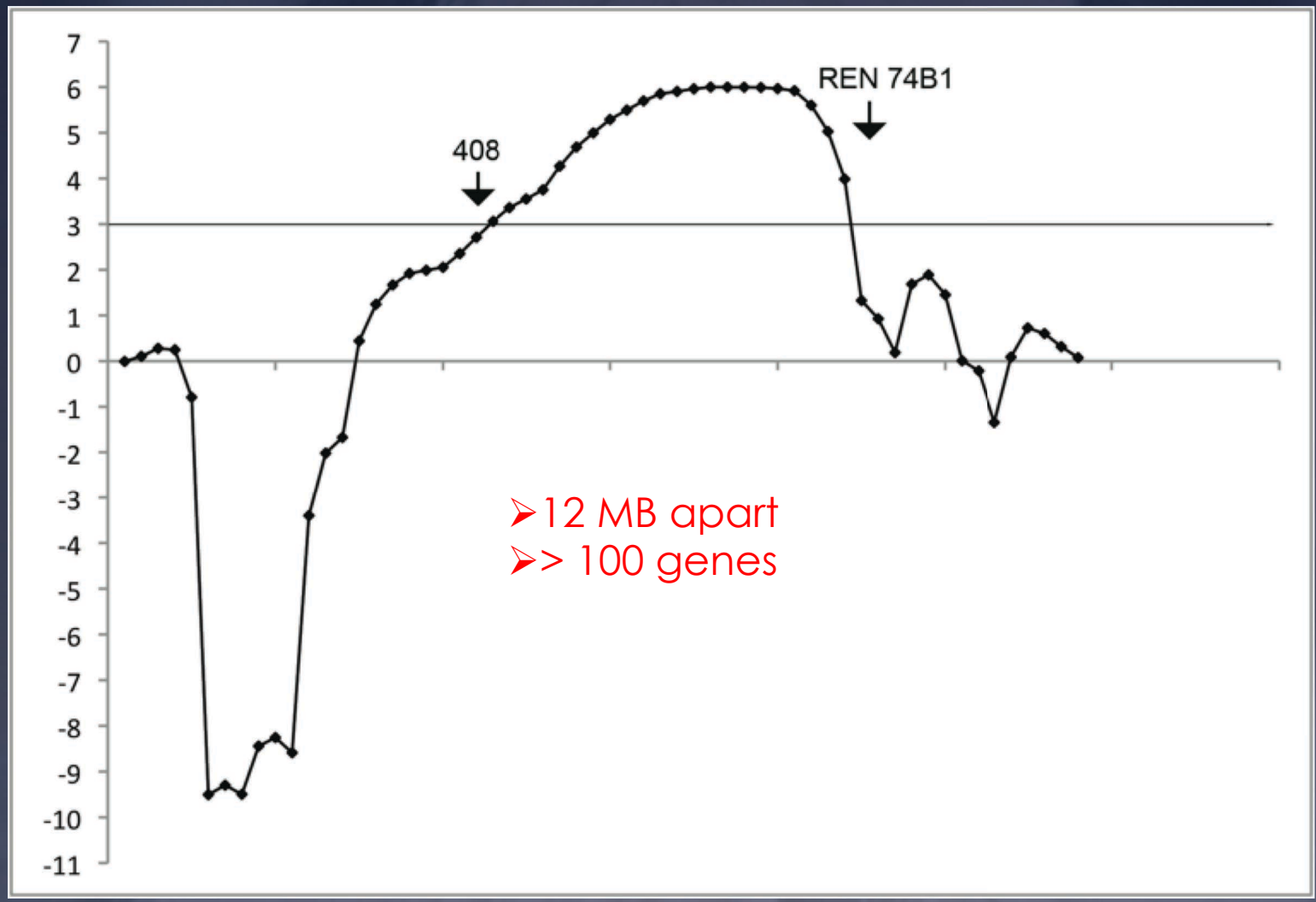
2006/8

OES Linkage study

- Genotyped a family of 48 dogs (Tonya Harris)
- Originally included 8 affected dogs.
- Over time added an additional 4 affected dogs



Linkage Analysis Results



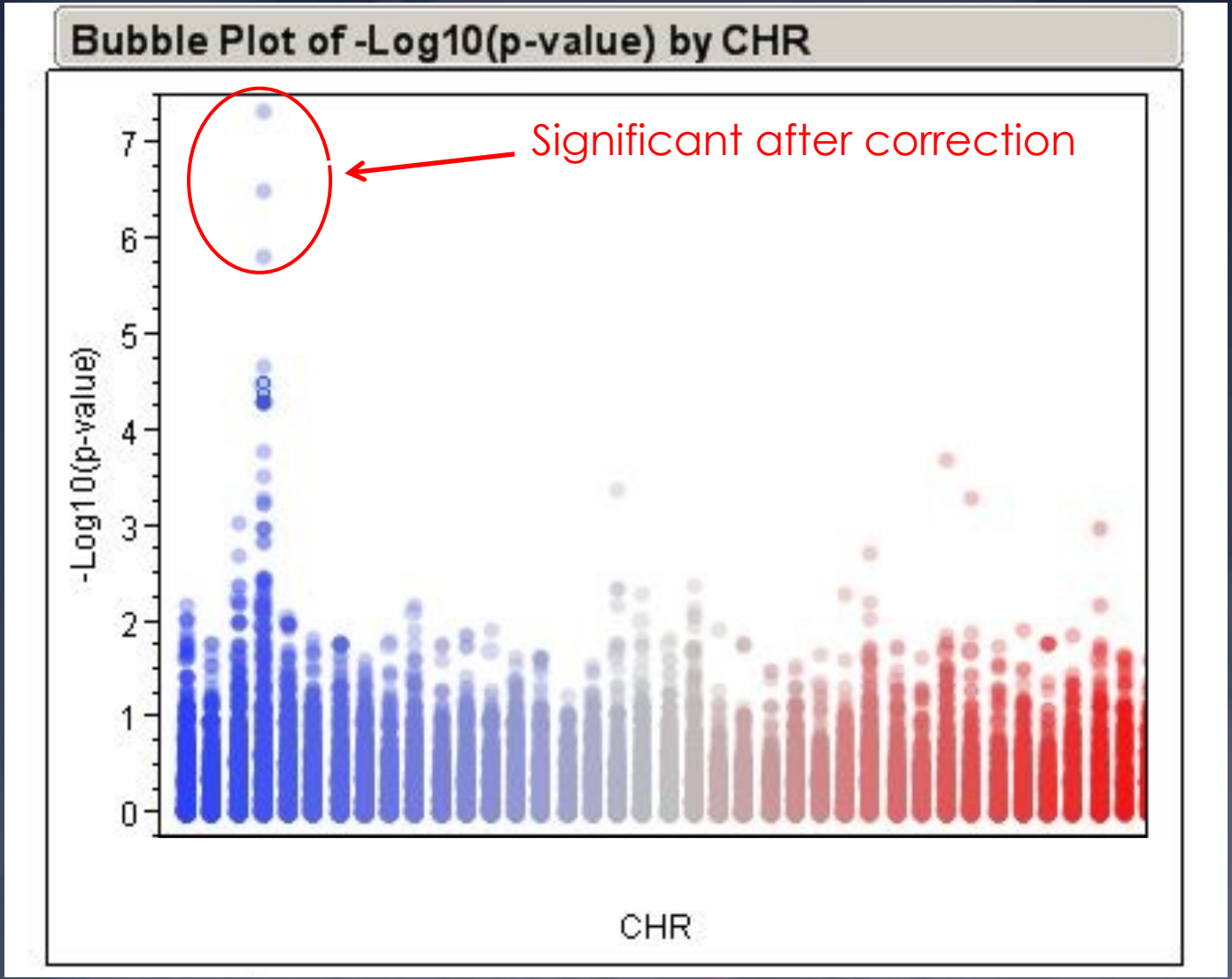
2008

Step 3: GWAS

- Genome wide association study will allow us to look at more markers in animals that are not necessarily related
- Used new chips from Illumina that allowed us to genotype 1000's of SNP markers at once
- Performed at Dr. Andy Singleton's Neurogenetics laboratory, NIH by myself and Cary Salzmann. Funded by Dr Singleton.

2010

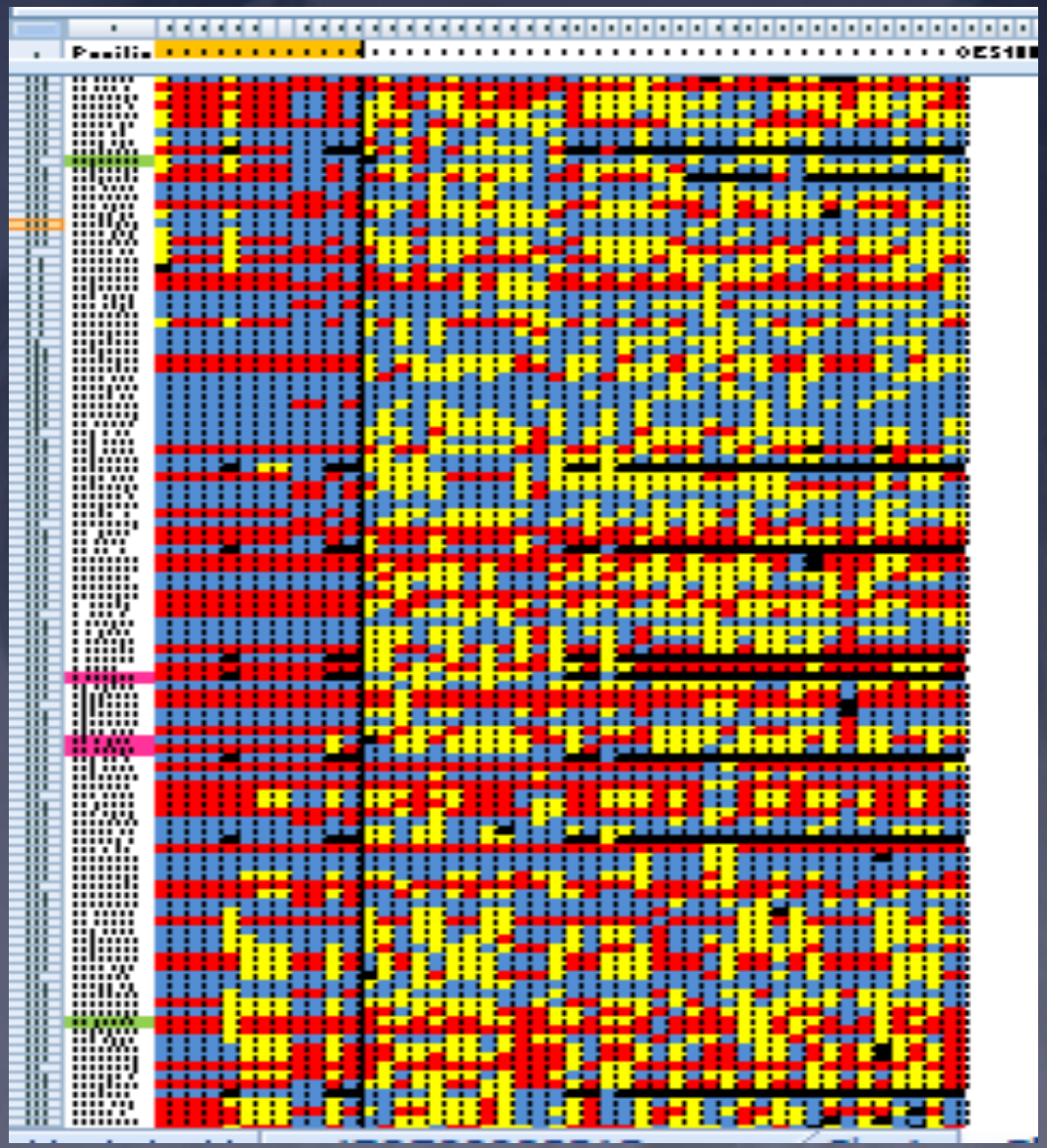
GWAS results



Cary Salzmann

Haplotype Analysis

* Red – homozygous
* Blue – homozygous
* Yellow - heterozygous

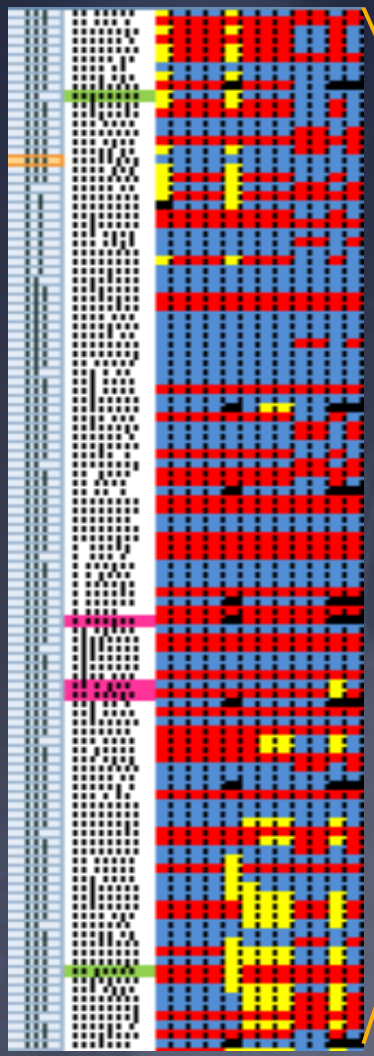


12MB Haplotype block

Natasha Olby

2011

Step 4: sequence capture



* New technology appeared and saved us!
* Sequenced the entire 12MB region in 3 normal and 3 affected dogs

Cary Salzmann, Dr. Noriko Tonomura, Dr. Kerstin Lindblad-Toh's laboratory at the Broad Institute

Cary Salzmann

Data Analysis

>3000 changes found!

Exomic variants only (coding regions)

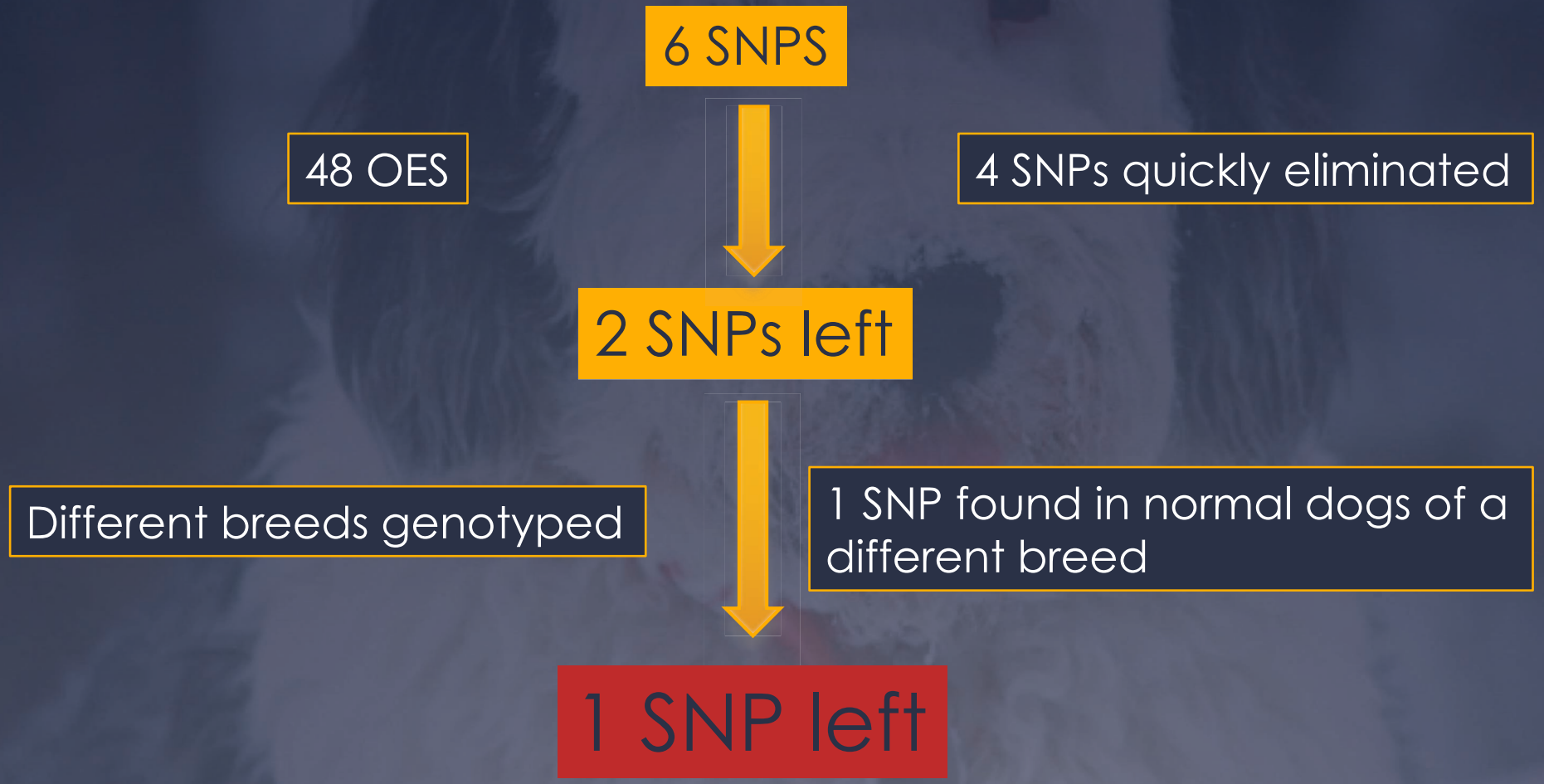
Fit autosomal recessive mode of inheritance

Non synonymous: change the protein

6 SNPs

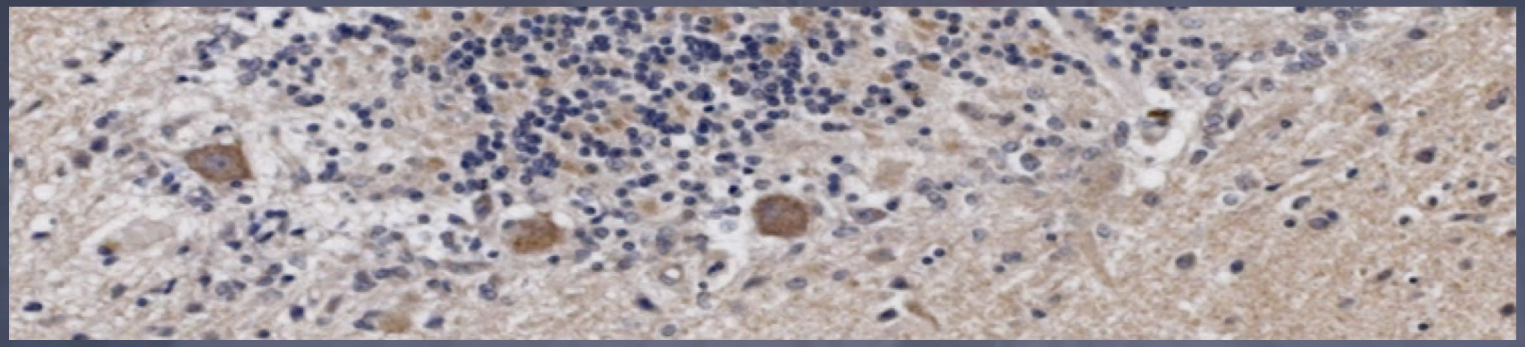
What now? .....Step 5

* Sequenced 6 SNPs with Sanger sequencing



Is this mutation the cause?

- Protein function predictors predict the mutation is malignant
- The protein is an enzyme important in autophagy
- Genotyped ~600 OES and this mutation is the only one associated with the disease
- Screened other breeds and the only one to have this mutation is the Gordon Setter

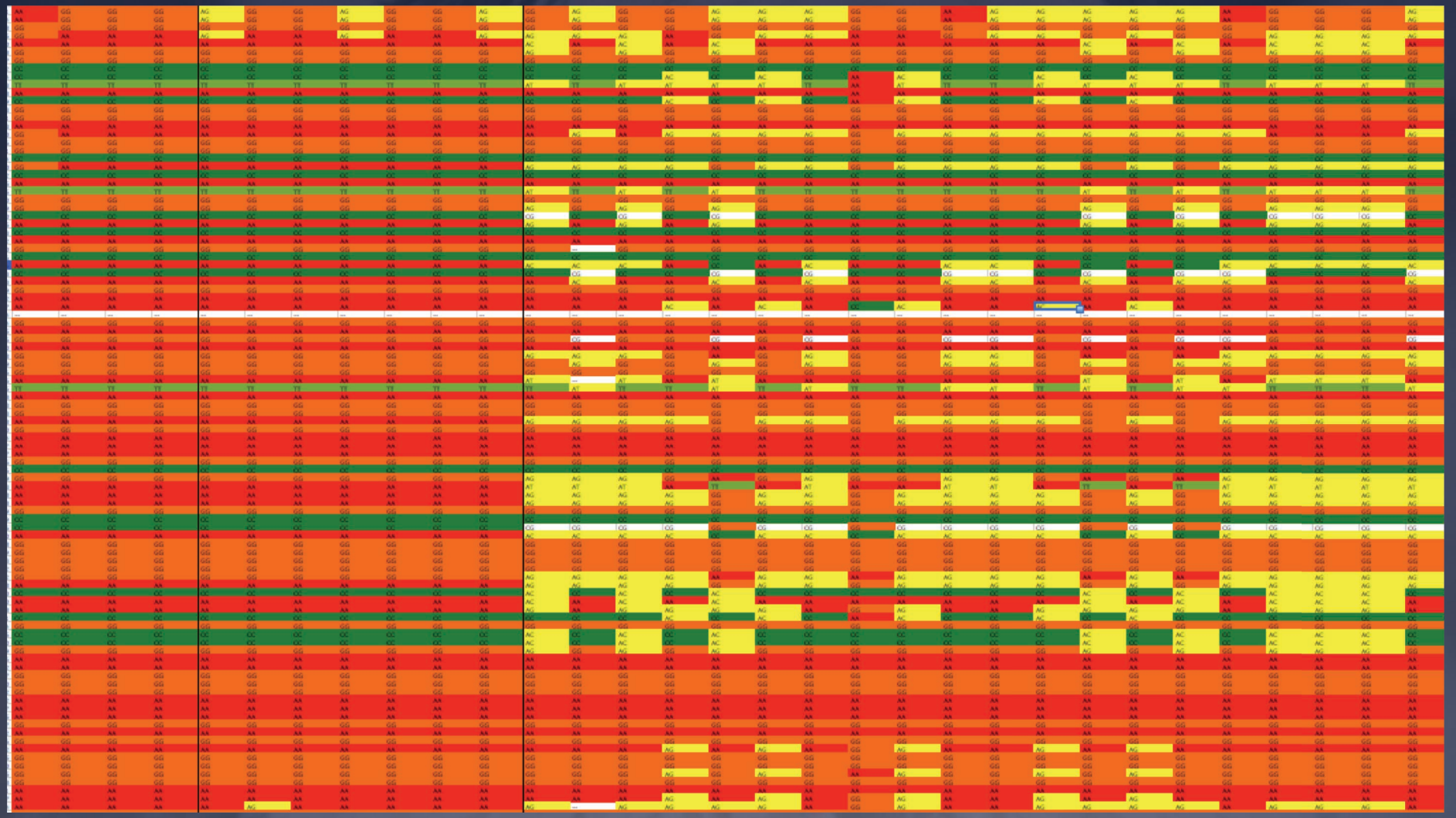


The Gordon Setter Story

- Have cerebellar degeneration with an almost identical phenotype
- All affected dogs were homozygous for the mutation
- Highly unlikely that the same mutation would be present in 2 breeds of affected dog by chance!

Gordon Setters and OES?

Gordon Setters and OES?



Important Facts

- We have a genetic test but how good is it?
- We have screened dogs from all major breed groups and it is not found in any other breed

	Old English Sheepdogs	Gordon Setters
Sensitivity	100%	100%
Specificity	99%	100%

- These numbers may change as we test more dogs
- 4 OES tested positive but were reportedly normal

More Important Facts

- How prevalent is the disease and the allele?
- Fortunately it is not very prevalent although numbers will change with more widespread testing

	Test population	Random population
Disease prevalence	6.25%	3.8%
Allele Prevalence	20%	14%

Can you test for it now?

* We hope to offer the test from next week
* Offered through NCSU Veterinary Genetics Laboratory (Dr Meurs)
* Cost: $51
* Link: http://www.cvm.ncsu.edu/vhc/csds/vcgl
* Can test from oral swabs or blood
* Turn around time is typically a week, results only given by mail and only to the person who sent in the sample

Ongoing Work and Plans

- Trying to prove causality
- Trying to understand how the mutation produces neuronal death
- Screening humans with hereditary ataxia for the mutation
- Aiming to submit publication in the next month

Acknowledgements

* Dr. Jerold Bell (Tufts)
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* Dr. Andy Singleton (NIH)
* Drs. Kerstin Lindblad Toh and Noriko Tonomura (Broad Institute)
* Drs. Dahlia Nielsen and Alison Mottsinger-Reif (NCSU Statistics)
* Dr. Ganokon Urkasemsin
* Cary Salzmann Agler
* Tonya Harris
* Pragna Mehta
* Kim Williams
* Dr Kate Meurs