

Cerebellar Degeneration in American Staffordshire Terriers: Finding the Abnormal Gene

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What is Cerebellar Abiotrophy?

- Neurodegenerative disorder affecting a part of the brain called the cerebellum
- Also called cerebellar ataxia, cerebellar degeneration
- There is a similar group of diseases in humans called
 - *the hereditary ataxias*
 - *the spinocerebellar ataxias (SCA)*

What is the cerebellum?

- Part of the brain that controls rate, range and force of movements

Nose



Back of
head

Signs of Cerebellar Disease

- Ataxia: incoordinated gait characterized by ‘hypermetria’ - overstepping.



Signs of Cerebellar Disease

- Wide based stance
- Intention tremor
- Nystagmus
*(uncontrolled
flicking of the eyes)*



Diseases of the Cerebellum

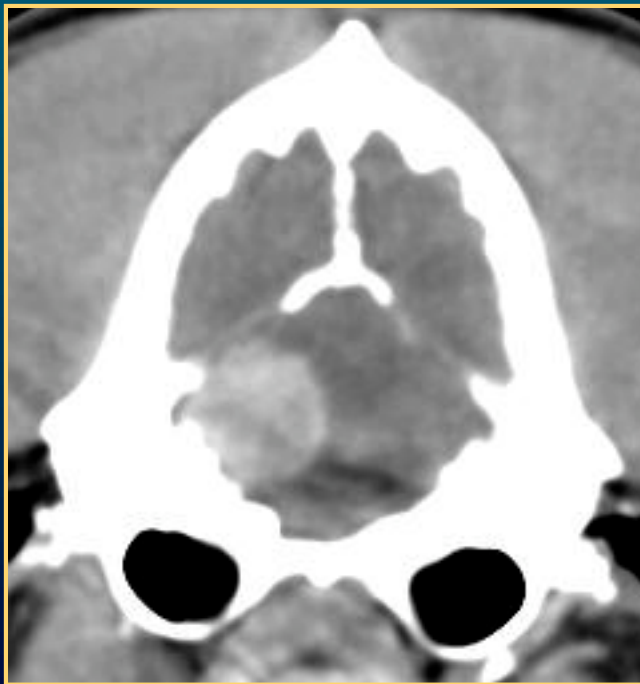
- Congenital anomaly - a malformation of the brain that is present from birth
- Encephalitis - infection or inflammation of the brain
- Brain tumor
- Stroke
- Toxicity - metronidazole at high doses
- Neurodegenerative disease (abiotrophy)

Diagnosis of Cerebellar Diseases

- Accurate history
- Careful physical & neurological examination
- Blood work, measurement of blood pressure, examination of the back of eye (fundic examination)
- Chest radiographs (older animals)

Diagnosis of Cerebellar Diseases

- Imaging of the brain - CT or MRI
 - *Will diagnose brain tumors, congenital anomalies, and in the case of MRI, stroke and degenerative diseases*



Diagnosis of Cerebellar Diseases

- CSF analysis - spinal tap
 - *Will allow the clinician to diagnose encephalitis*
 - *Performed under general anesthesia*



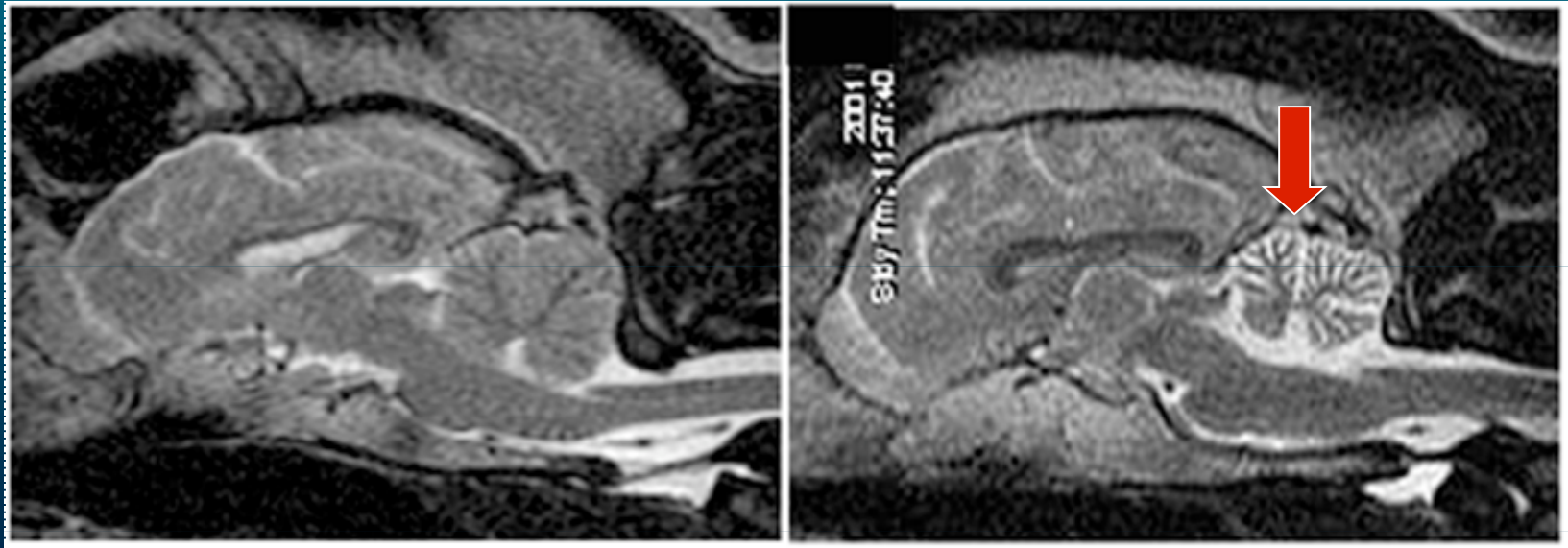
What do we know about CA in dogs?

- It has been reported in multiple breeds, usually with onset of signs at an early age.
- Five breeds have a later onset disease
 - Brittany spaniel
 - Gordon Setter
 - Old English Sheepdog
 - American Staffordshire terrier
 - Scottish Terrier

What do we know about CA in dogs?

- History of slowly progressive signs of cerebellar disease
- Blood work and general physical examination is normal
- CSF analysis is normal
- MRI shows atrophy of the cerebellum

MRI Findings



Histopathological findings

- Cerebellar atrophy noted on MRI is obvious at necropsy:

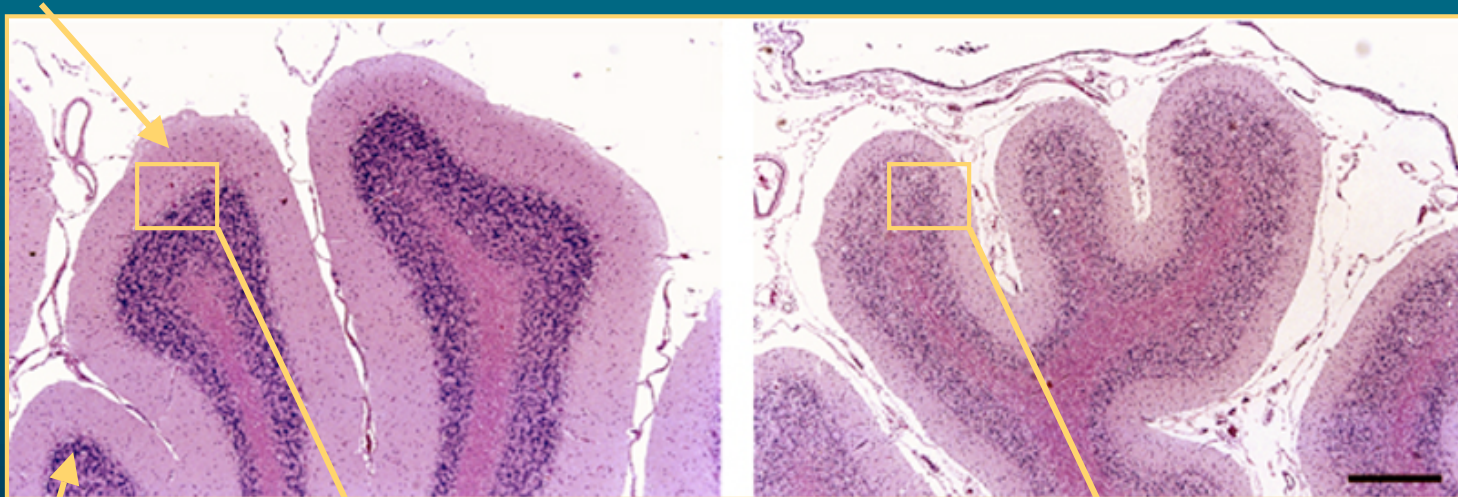


Histopathological findings

Molecular layer

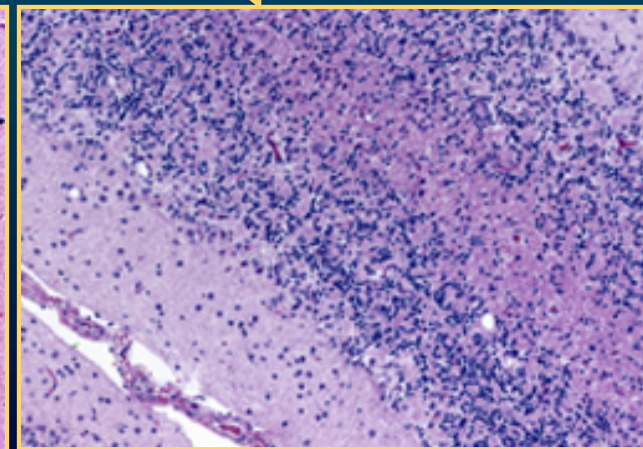
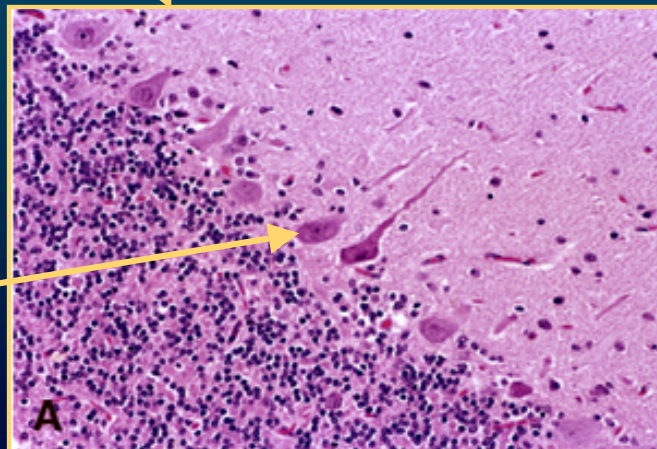
Normal

Affected



Granular layer

Purkinje layer

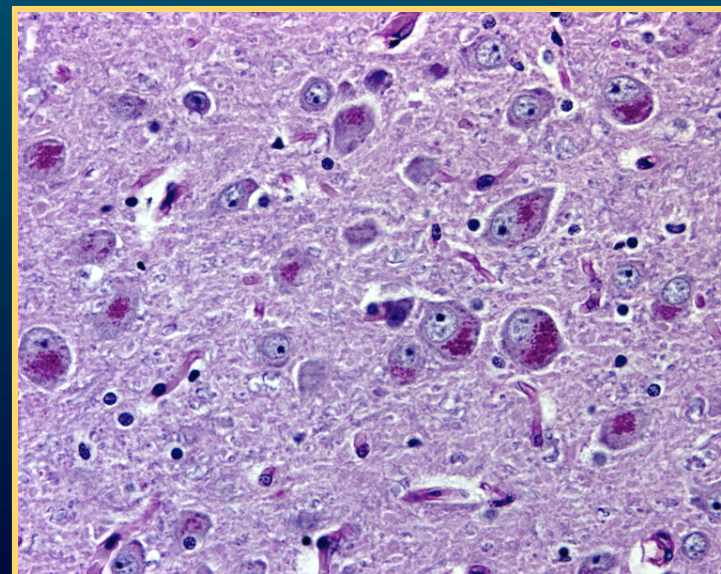
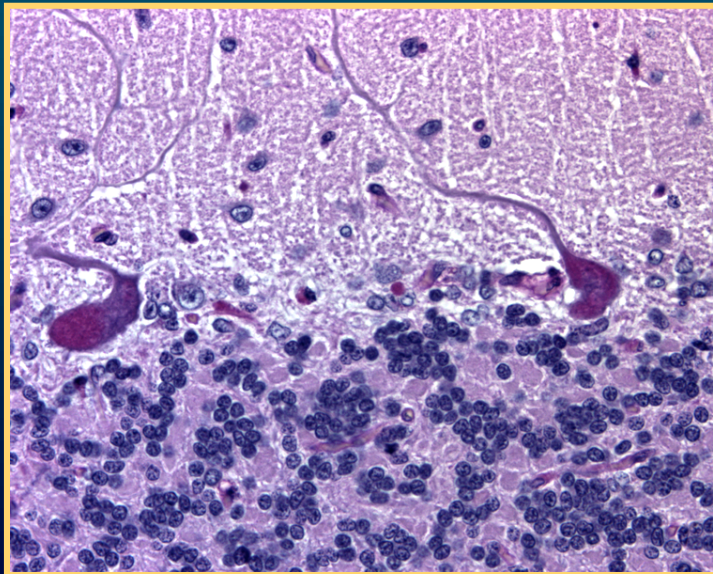


What do we know about CA in AmStaffs?

- Onset of signs between 3 and 8 years of age
- Notice difficulty with stairs and corners
- May run into things
- When they jump up on back legs, tend to go stiff and fall over backwards
- Hind limb gait becomes stiff and they may bunny hop and spring like a lamb
- Notice nystagmus and ‘ocular dyskinesia’

CA in AmStaffs

- Progress to an inability to walk over a period of years.
- The earlier the onset of signs, the faster the progression.
- Ceroid accumulations in the thalamus & in the Purkinje neurons only - not typical of a storage disease

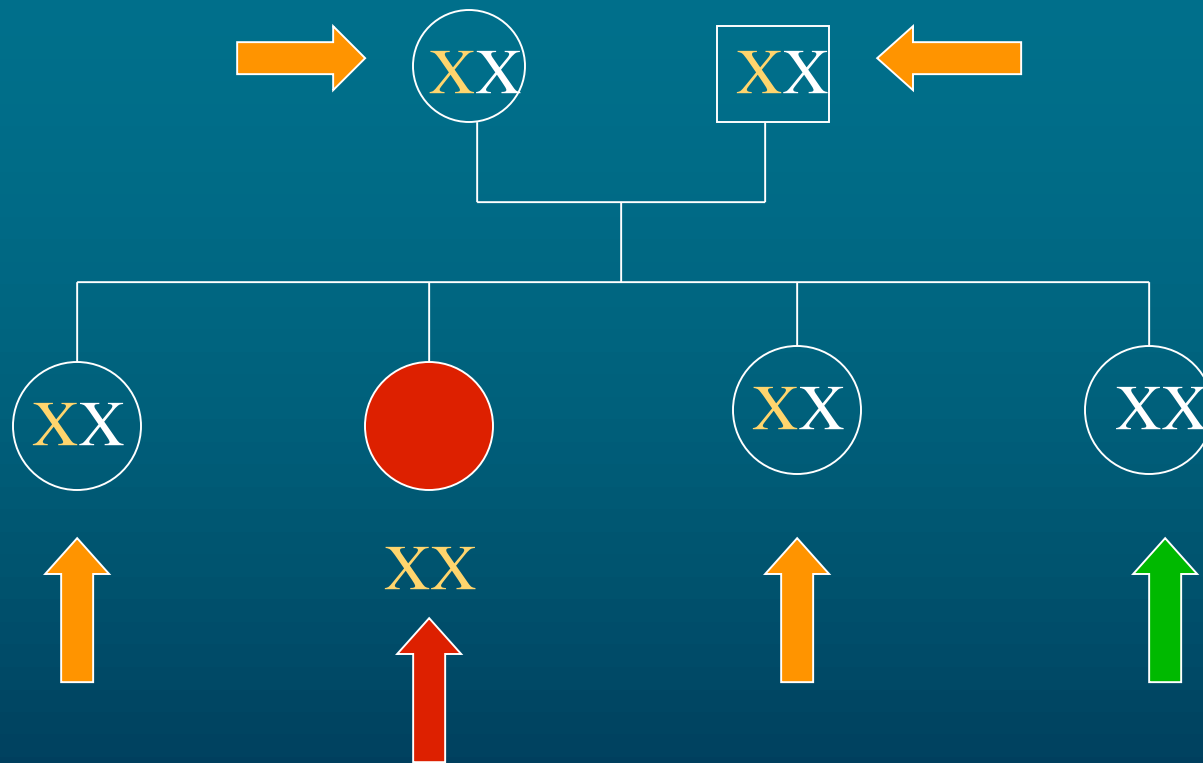


CA in AmStaffs

- It is hereditary.
- Inheritance - most consistent with autosomal recessive.
- Conservative estimate: 1 in 350 registered dogs affected.
- Abnormal allele is present in approximately 40% of dogs.

Autosomal recessive diseases

- Need 2 copies of the abnormal gene to show signs of the disease, one from each parent
- If have only one copy of the gene, will not show any signs - silent carrier



Affected



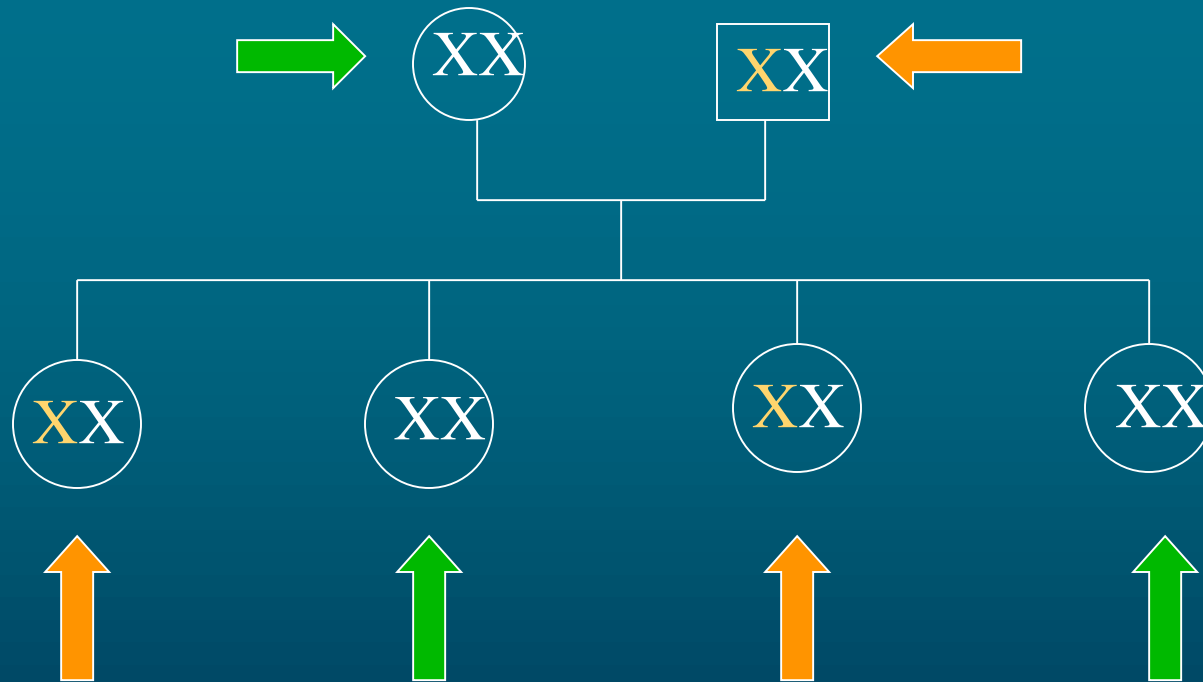
Normal carrier



Normal

X: abnormal (mutant) gene

X: normal gene



Affected



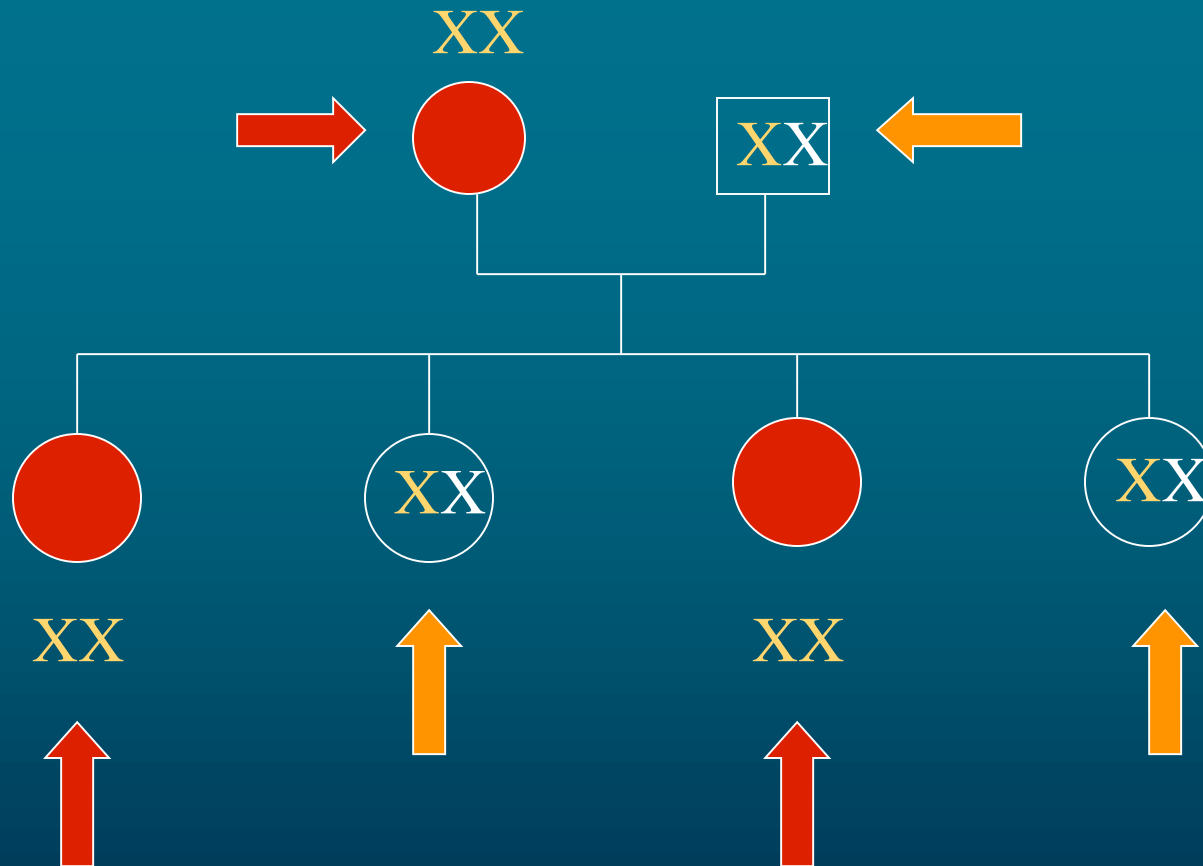
Normal carrier



Normal

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Affected



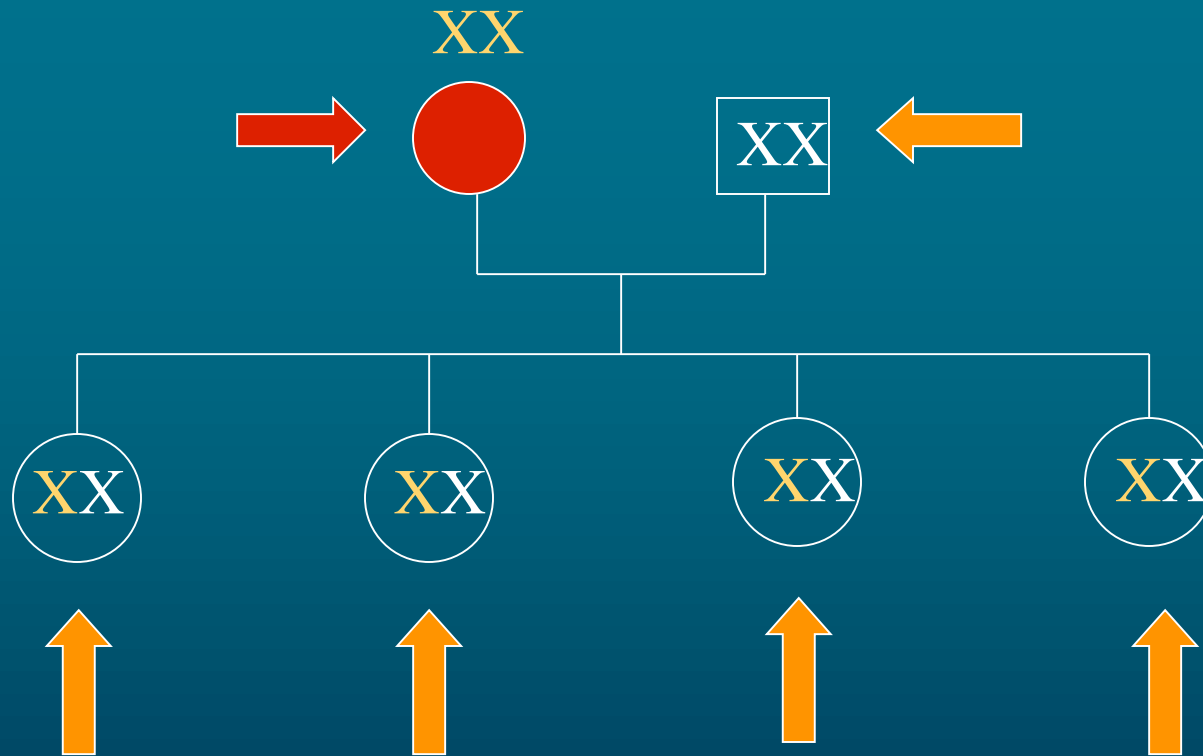
Normal carrier



Normal

X: abnormal (mutant) gene

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Affected



Normal carrier

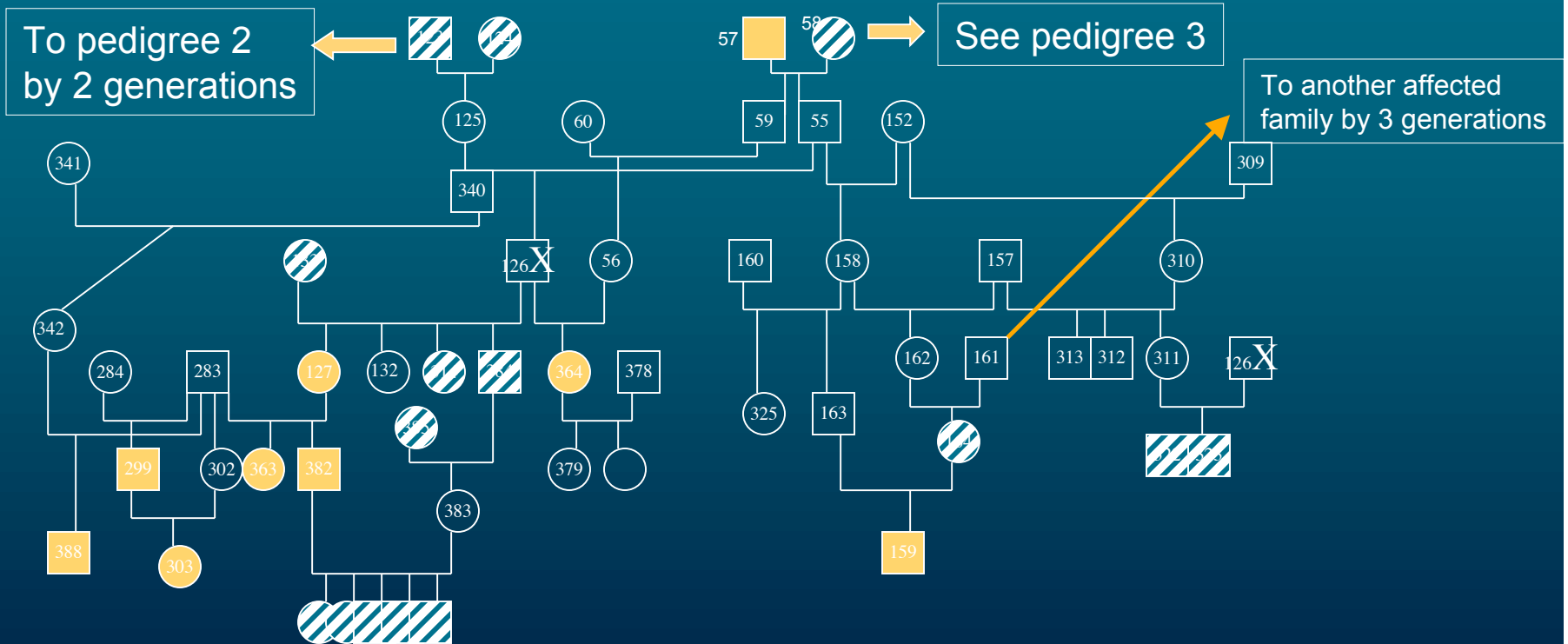


Normal

X: abnormal (mutant) gene

X: normal gene

Example family



How do we find the abnormal gene?

1. Describe the disease in detail (Olby et al., 2004)
2. Determine the mode of inheritance
3. Collect DNA from families of affected dogs
 - Determining phenotype (affected or normal)
 - Maintaining anonymity
 - Actual sampling procedure

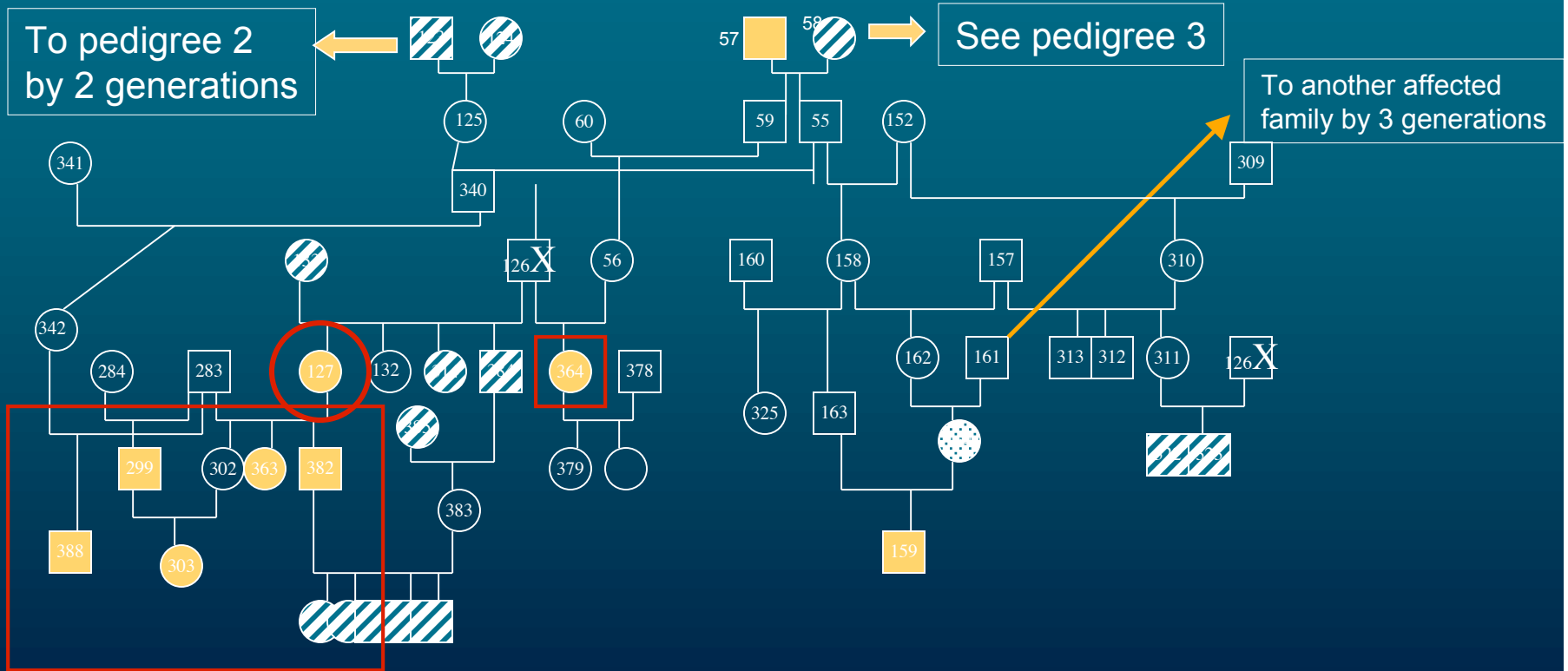
Establishing Affected Phenotype

- Expensive for owners to complete a full workup.
- At the time of euthanasia, many owners are unwilling to have a necropsy (autopsy) performed to confirm a diagnosis.
- Consistent long history and clinical signs are very suggestive of the disease:
 - in my experience every dog that I have followed to final autopsy has been correctly diagnosed prior to death.
 - www.acvim.org Find a specialist

Affected Phenotype Guidelines

- My approach to affected phenotype is:
 1. *Gold standard: necropsy confirmation*
 2. *Next level: compatible history, full workup with a neurologist (MRI or CT and CSF analysis).*
 3. *Also excellent: close relative of confirmed case.*
 4. *Considered likely: compatible history and clinical signs*
- Over time dogs tend to move up from category 4 to 3 if necropsies of relatives are obtained
- The dog owners and researchers must remain in contact in the long term

Example family



Normal Phenotype Guidelines

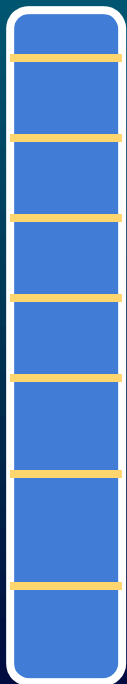
- The dog must be normal beyond the age of the latest reported onset of the disease
 - > 9 years of age.
 - Accept dogs of > 6 years of age
 - Collect from dogs directly related to affected dogs at any age, but I may have to hold the samples for a long time.

Finding the Gene: Candidate Gene Approach

- Identify similar diseases in the same or different species for which the mutation is known.
e.g. www.geneclinics.org/profiles/ataxias/details.html
- Amstuffs have an usual accumulation of a lipid (ceroid) in specific groups of neurons - nothing quite like it reported previously
- We looked at and ruled out
 - A disease caused by vitamin E deficiency
 - A calcium channelopathy

Finding the Gene: Linkage Analysis

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

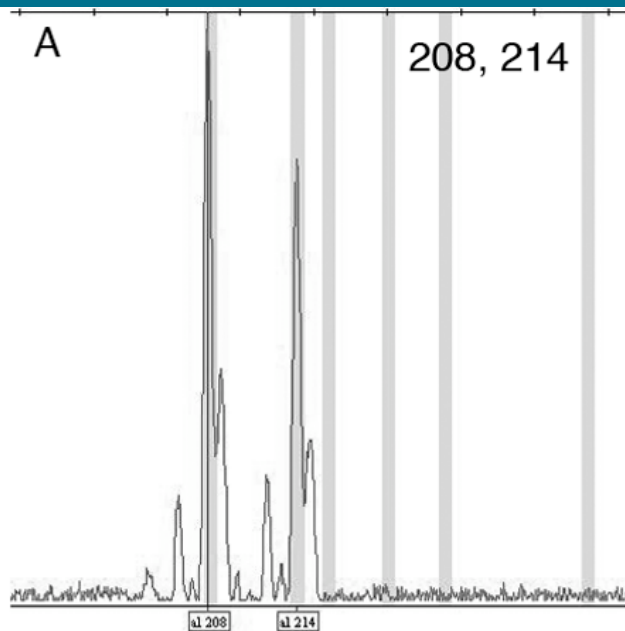


Each 'marker' has been selected because there are several different versions - alleles

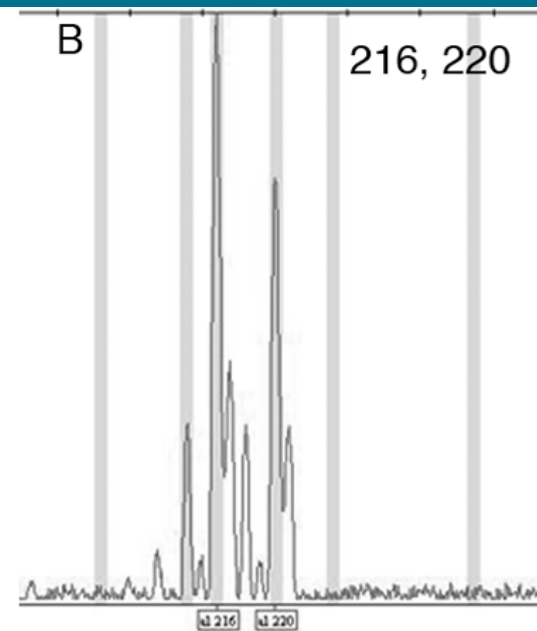


Each allele will be passed from parents to offspring and can be traced down the generations

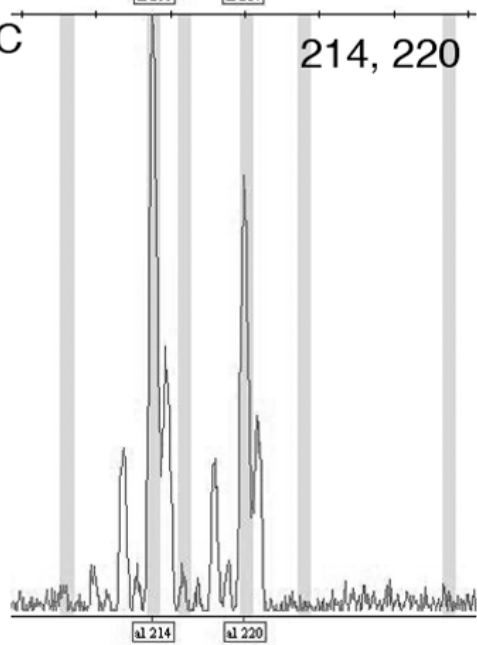
A 208, 214



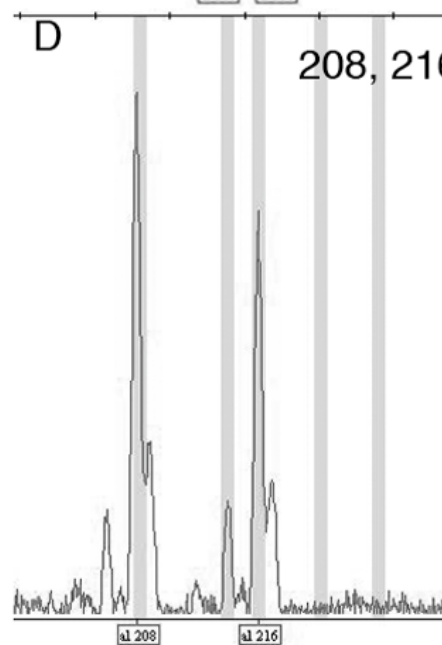
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C 214, 220

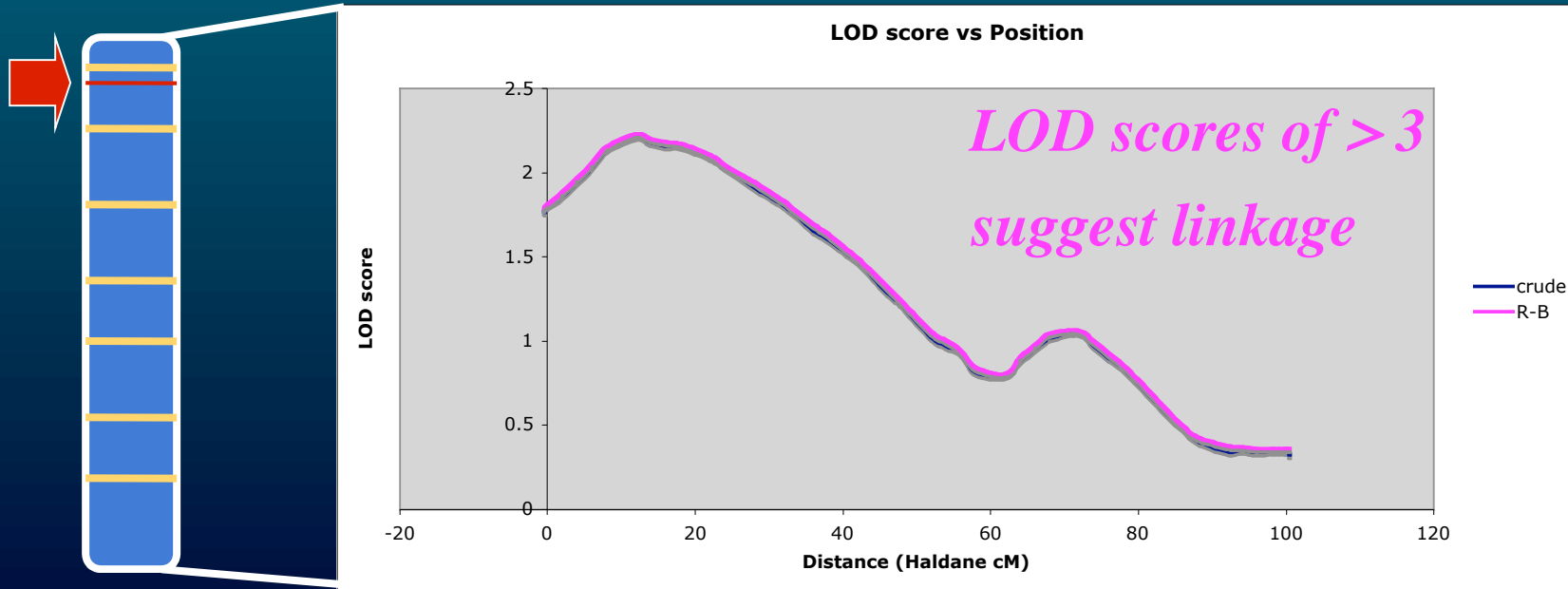


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Finding the Gene: Linkage Analysis

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



Finding the Gene: Linkage Analysis



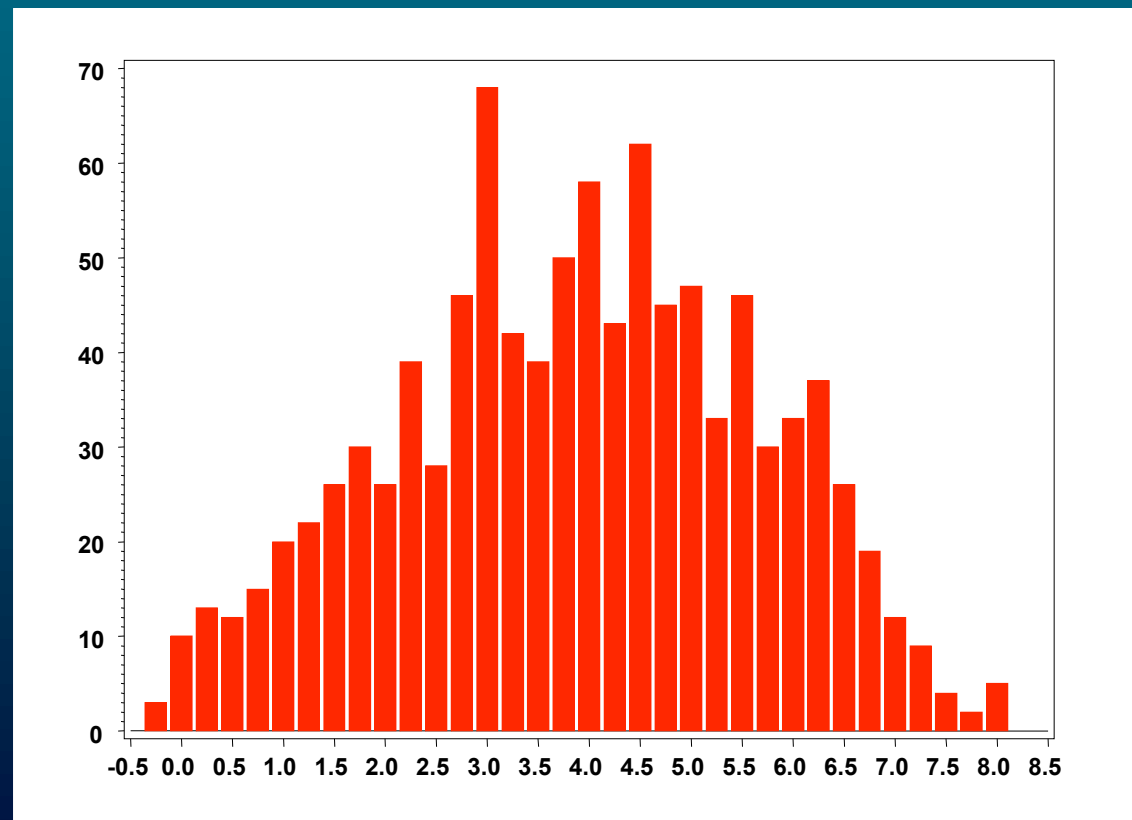
- Once you have linkage....you are simply linked to a chromosomal region, not a single gene.
- We then look at the published information on the canine and human genome maps to see if there is a good candidate gene in that area.
- If there is not, we saturate the area with markers closer together and repeat the linkage analysis.

How do you know whether linkage analysis will work

- It is important to simulate a linkage study prior to starting it, to see if there is a statistically good chance of finding linkage.
 - We use 315 markers for each dog: this represents an enormous amount of labor and costs about \$500 in consumables per dog
 - The DNA is precious: we don't want to use it until we have a good chance of getting the answer

How do you know whether linkage analysis will work: results of simulation

Frequency of lod score



Lod score

Stages involved in linkage analysis

Collect DNA and pedigree information



Markers cost: \$30,000

Perform PCR reactions using the markers

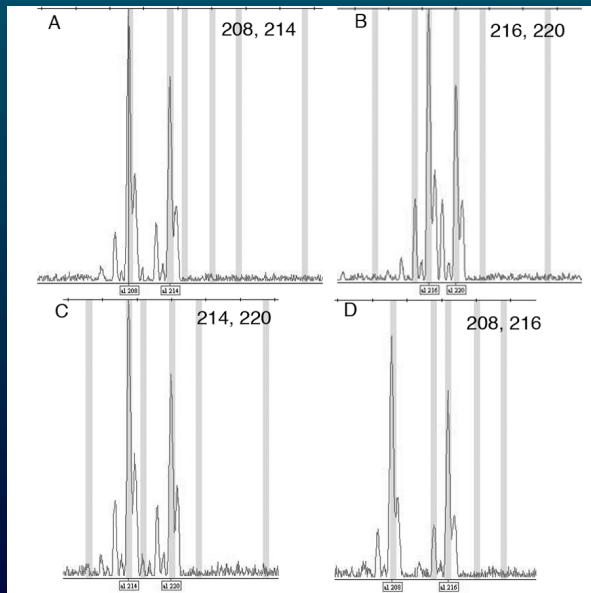


Reagents cost: \$300/dog

Generate electropherograms



Plates cost: \$200/dog



Use genemapper to pick alleles



Software cost: \$10,000

Send pedigree information & allele picks to Dr. Dahlia Nielsen: linkage analysis

Ways in which it can go wrong

- Phenotype incorrect
- Dog identity incorrect
- Inadequate statistical power
- Uninformative markers (high level of homozygosity)
- Gene not linked to marker
- Incorrect allele picks

Progress on the project

- We spent the summer of 2005 getting the multiplex (marker) sets to work consistently.
- We have nearly completed the genome screen of about 60 related Amstaffs
- We have shown that several chromosomes are NOT linked to the disease
- We have linkage on one chromosome that we are currently pursuing
- We are still completing the analysis of full families

What will we have by March 2007?

- Lod scores for all of our markers
- If we are lucky....one of them will be very high and will lead us to the correct chromosomal region
- Realistically, we may have several different markers with high enough lod scores that deserve further evaluation
- We will then start the process again focusing on these regions.

What will we have by March 2007?

- There are many unknowns making it impossible to predict how long this will ultimately take and whether we will be successful
- New, faster and more accurate techniques continue to appear - don't be surprised if our plan changes with the times.

My lab.....

- Pragna Mehta extracts DNA and is a point of contact for owners and veterinarians.
- I construct pedigrees and go on blood draw field trips.
- The endless lab work is done by Pragna Mehta and Tonya Harris
- Allele calls are made by myself, Sara Gray (summer student) and Tonya Harris
- Dr. Neilsen and Rachel Myers do the linkage analysis
- *Matthew Breen and Rachel Thomas provide us with constant advice.*

My lab.....

At any one time approximately 3 people are working on this project

The grant pays for a half time technician only

What can you do to help?

- Please contact me if you have an affected dog - see protocol sheets on the health page of the STCA web site.
- If you have a dog directly related to an affected dog, please contact me.
- If you can make a donation, please let the health committee or me know: every penny counts at this point.
- Be patient if we do not get right back to you: I receive hundreds of e mails and phone calls a day.

Questions I have been asked

1. Is a genetic test accurate?
 - YES
 - Once developed, it accurately detects affected and carrier status (like a DNA test for parenthood).
 - Please note that in humans there are genetic tests for diseases like Alzheimer's disease. You can be negative for this test and still have Alzheimer's disease.
 - This is because there are both hereditary and non hereditary forms of Alzheimer's.
 - Only one form of CA has been identified in Amstaffs at this time and we are interested in the hereditary forms of CA only for breeding purposes.

Questions I have been asked

2. How long will it take to find the mutation and develop a test?
 - I don't know. The genome screen should be complete and linkage analysis performed by the end of the granting period.
 - I can't guarantee that we will find the mutation

Questions I have been asked

3. If people contact me will their information be kept confidential?
 - YES

4. What do I think about an open registry?
 - A genetic test would provide a much better solution
 - Selective breeding based on assessment of pedigrees without knowing the true genetic makeup of each individual may lead to exclusion of excellent breeding dogs
 - Responsible breeders will not knowingly breed dogs known by them to be carriers.

My experience so far.....

Amstaff breeders are the BEST! They have been very open and helpful to me, allowing me to collect DNA from closely related families of dogs over several generations.