

## Atopic Dermatitis in West Highland White Terriers: Linkage Analysis

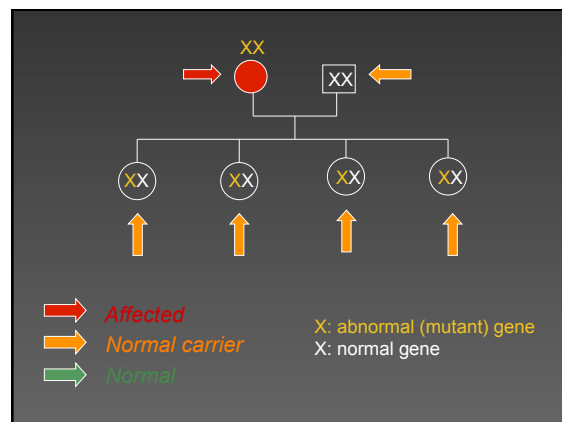
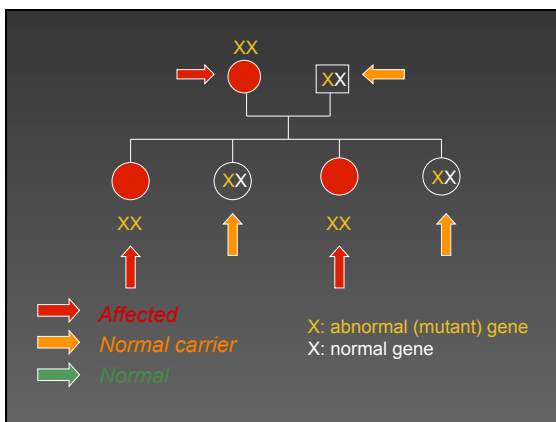
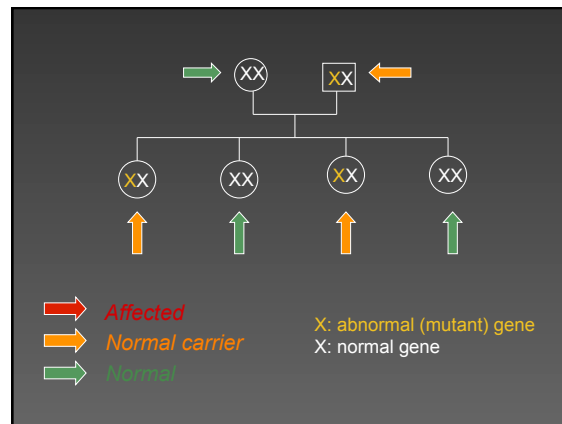
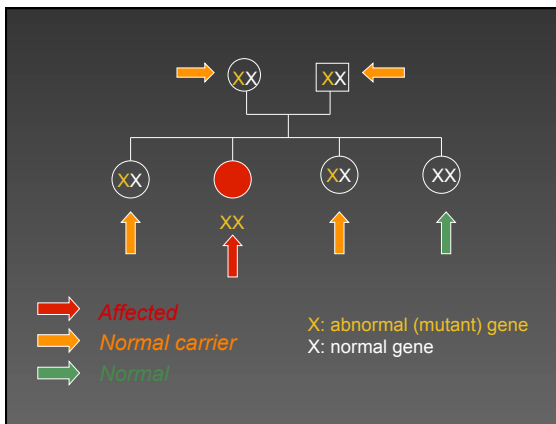
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## Mode of Inheritance

- Information was collected on full litters containing affected dogs
- A Chi squared analysis was performed to compare expected and actual numbers of affected dogs
- Results were consistent with a **simple autosomal recessive** inheritance



## How do we find the abnormal gene?

1. Collect DNA from families of affected dogs  
(Acorn grant funded by AKC CHF in 2004 for \$13,000)
  - Determine phenotype (affected or normal)
  - Maintaining anonymity
  - blood samples

## Establishing Phenotypes

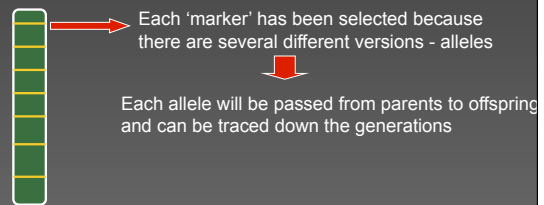
- Atopic dermatitis:
  - clinical signs compatible with atopic dermatitis
  - juvenile and adult dogs
  - often family history of allergic skin, eye or respiratory diseases
- Normals:
  - No history of skin diseases or allergies
  - > 4 years of age

## 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](http://www.geneclinics.org/profiles/ataxias/details.html)
- In humans, mutations in filaggrin is an important cause of the disease.
- Another group has ruled-out filaggrin mutations in AD in westies

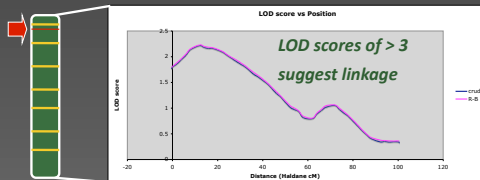
## Finding the Gene: Linkage Analysis

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



## 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*



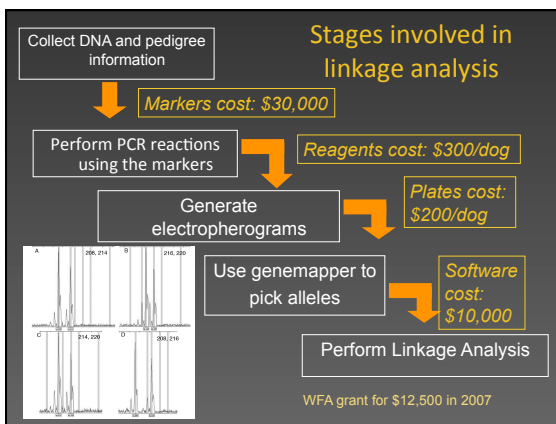
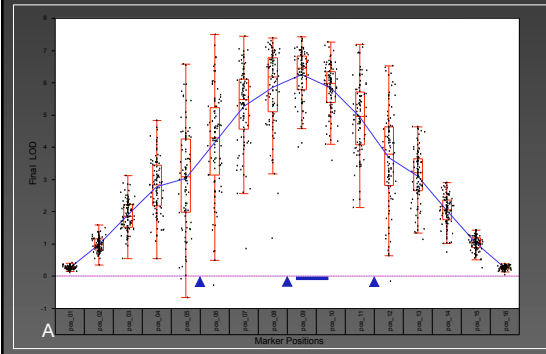
## Finding the Gene: Linkage Analysis

- 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

## Simulations performed by Dr. Dahlia Nielsen



## 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
- Strong influence of environment that could alter the true phenotype

## Results of Linkage Analysis

- 108 Westies: 67 used (30 affected, 50 normal, 24 "undetermined" status)
- We started with the chromosome that contains the filaggrin gene.
- At first we had a promising LOD score
- Addition of more markers and dogs lowered the LOD score.
- There were no significant LOD scores on any chromosome (no association of AD with any marker).

## What does this mean?

- There are two main reasons we could have failed to identify a linked region:
  - The causative mutation has a low effect on phenotype and our study did not have the power to detect it
  - The phenotypes could be incorrect – in particular the normals may not be normal
  - There could be a strong influence of the environment?

### What should we do now

- A genome wide association study with higher power using gene chips
- Need to test at least 200 dogs
  - Sep 1, 2010: 61 Affected Westies
  - 44 Normal Westies
  - 37 Affected other breeds (16 different breeds)
    - WFA provided \$5,000 for shipment costs
- Funding status:
  - Budget: \$144,000/2 yrs (reagents and salary support)
  - \$5,000 received from WFA for sample shipment
  - No funding from NIH, AKC-CHF, Morris Animal Foundation
  - Establishment of an international collaboration with European groups to increase chances of funding?

### NCSU Research Team

- **Genetics/Neurology:**
  - Dr. Natasha Olby – oversees the work
  - Dr. Dahlia Nielsen – geneticist and statistician – wrote all scripts, performed simulations
  - Cary Salzmann – PhD student: completed linkage and pedigree analyses
  - Tonya Harris – research technician – performed the PCRs and linkage analysis
- **Dermatology:**
  - Dr. Thierry Olivry – clinical partner (verifies phenotypes)
  - Judy Paps – research technician – supervised first set of sample collection