



# **Addison's & the Bearded Collie**

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# Goals of Any Breeder

- **Maximize Desirable Traits**
- **Minimize Undesirable Traits**
- **Eliminate Health Disorders**

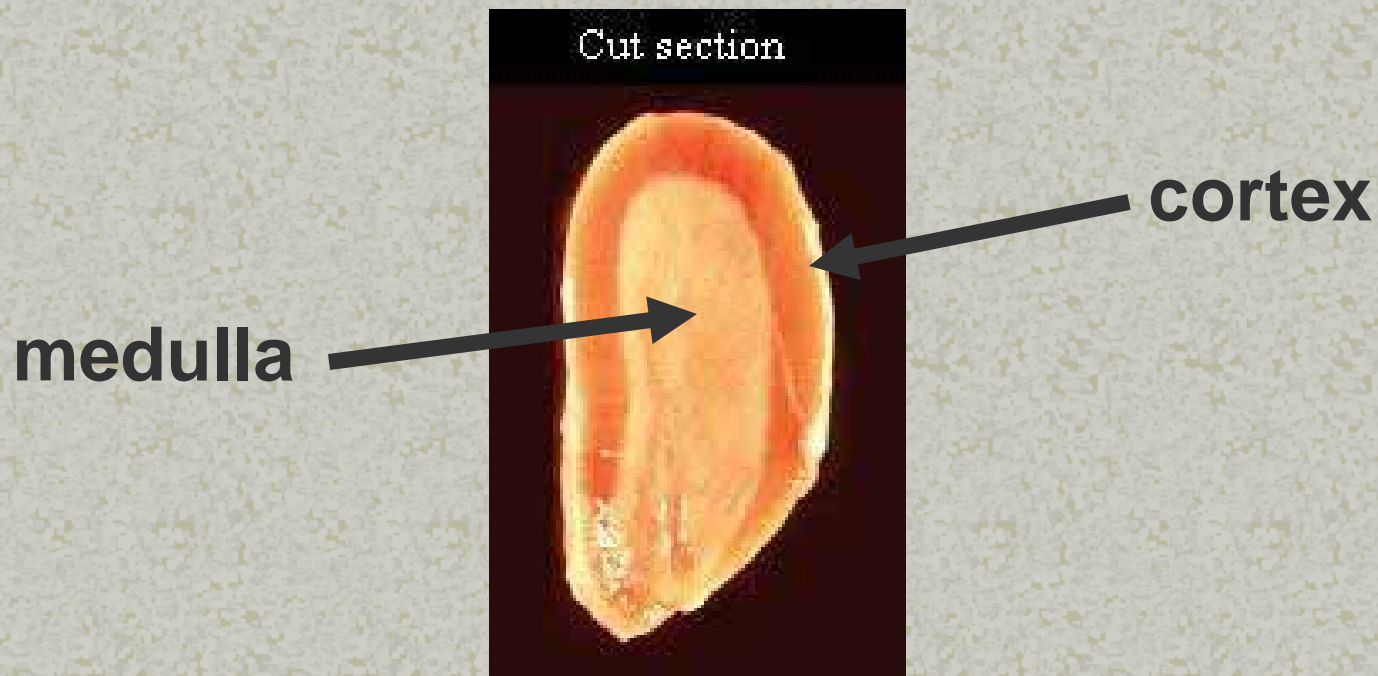


# **Addison's Incidence in the Bearded Collie**

- **Owners & Breeders concerned**
- **Reports in the Literature**
- **Addison's estimated incidence in Bearded Collies is 2-3.4% from health survey data done by club**

# What is Addison's Disease?

- Defined as a deficiency in the adrenal cortex to produce glucocorticoid and/or mineralocorticoid



# Adrenal Cortex Regulation

**Stressors**

**Blood changes**

Hypothalamus

*CRH*

Pituitary

*ACTH*

Adrenal cortex

*Angiotensin II*  
*Ion changes*

**Glucocorticoids**

(Cortisol)

**Mineralocorticoids**

(Aldosterone)





# What is Addison' Disease?

- Defined as a deficiency in the adrenal cortex to produce glucocorticoid and/or mineralocorticoid
- “Later onset” adrenal cortex failure (but age of onset can range from 6 months to 10 years old)
- Two main Categories
  - Primary Hypoadrenaocorticism
  - Secondary Hypoadrenocorticism



# Addison's Symptoms

- **Reflect the absence of the hormones:**
  - Inability to accommodate stress
  - Inability to regulate sodium and potassium levels
  - Altered metabolism
  - Lethargy
  - Gastric Disturbances: vomiting, diarrhea



# **So if Goals of Any Breeder is to.....**

- **Maximize Desirable Traits**
- **Minimize Undesirable Traits**
- **Eliminate Health Disorders**



# What Steps Needed to Achieve those Goals?

- Determine if the trait is heritable
- Breed to maximize or minimize heritable traits
- Develop rearing standards for those traits that are not heritable





# Basic Canine Genetics

- 38 pairs of chromosomes (autosomes) plus the X & Y (sex chromosomes)
- Chromosomes are segments of DNA
- DNA is made of repeated nucleotides or bases (abbreviated A, C, G, & T)
- DNA is the template of genes
- Dogs have about 3,000,000,000 bases of DNA in one set of chromosomes
- About 80,000 different genes

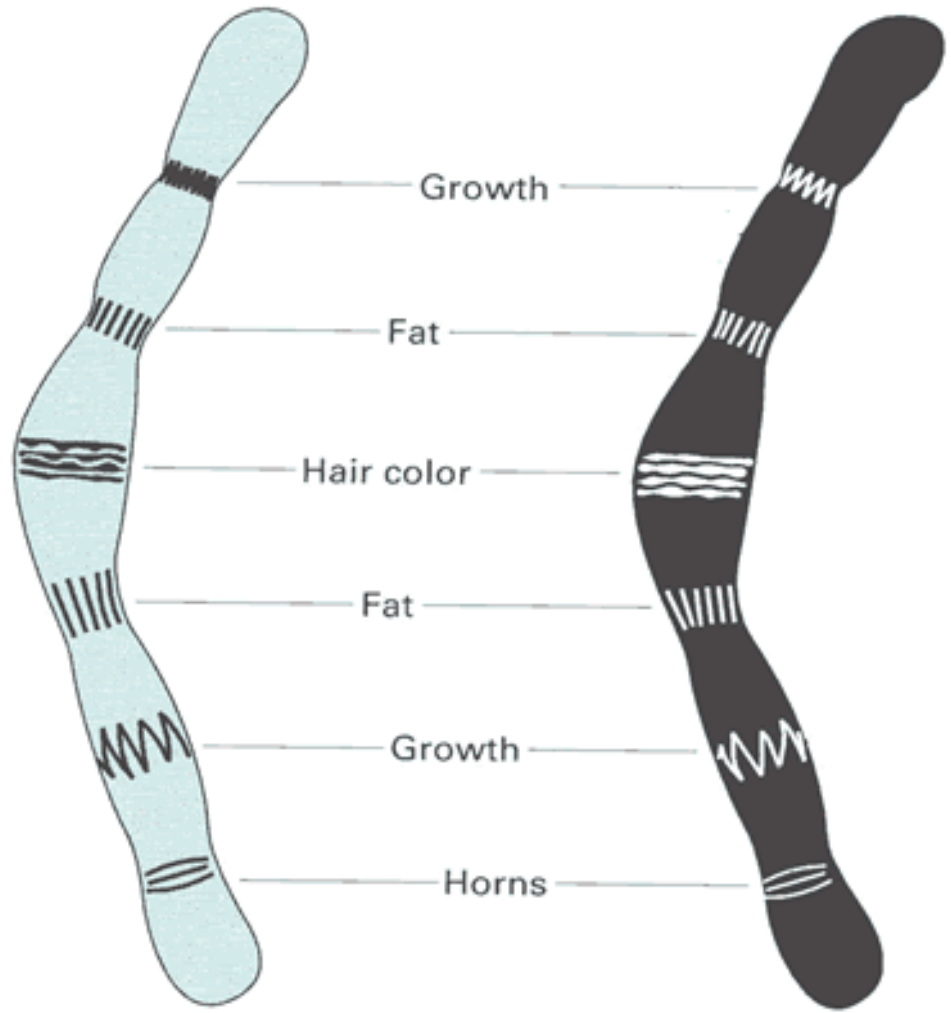


# Basic Canine Genetics (cont.)

- Genes are regions of DNA that specify/code for a protein product
- Each gene on an autosomal chromosome is present in two copies



Gene pairs for:



**Paired genes on chromosomes**



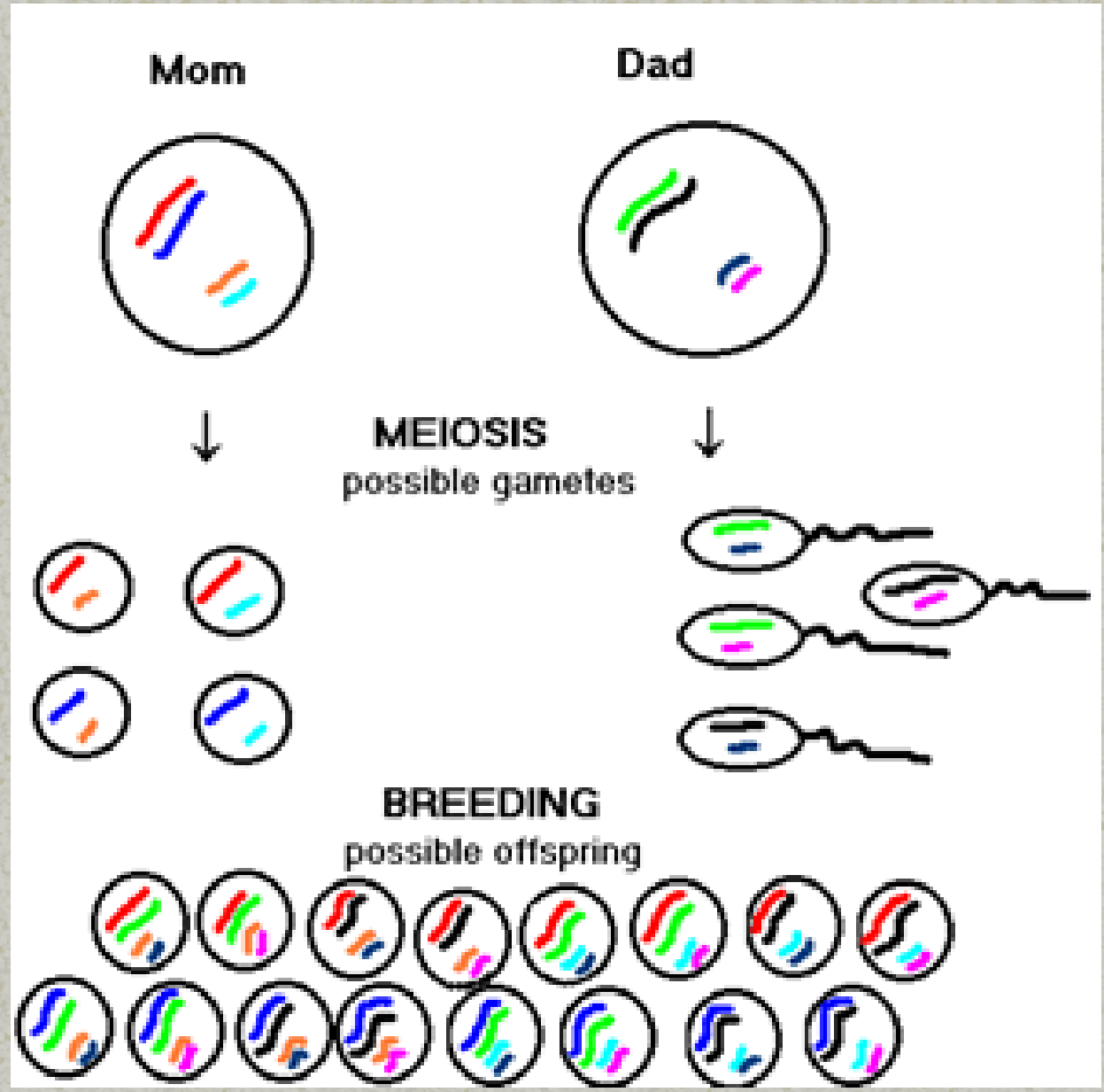
# Basic Canine Genetics (cont.)

- The different versions of the same gene are referred to as *alleles*
- Alleles are different versions of the same gene (long vs. short hair)
- Allele versions are due to changes in the underlying DNA sequence (e.g., a missing base causes PRA in Cardigan Welsh Corgis)



# Basic genetics cont. (still)


- **Homozygous:** the alleles for a given gene are *identical* on both chromosomes
- **Heterozygous:** the alleles for a given gene are *different* on both chromosomes (also called “carriers”)



# Why do statistical research?

- To determine if a trait is genetically controlled. A trait may be:
  - **Not genetic**





# Non Genetic Traits are those that are:

- Not regulated by genes
- Cannot be “selected” for in breeding programs





# **Non Genetic Traits**

- **Sunbleached fur**
- **Cataracts due to eye damage**
- **Accidental broken bones**
- **Urinary stones caused by bacterial infection**
- **Dyed skin**
- **Cropped ears**

# Why do statistical research?


- To determine if a trait is genetically controlled
  - Not genetic
  - **Genetic but not inherited (i.e., “somatic”)**





# Genetic but non-inherited traits are:

- Regulated by altered genes/DNA whose changes somatic and *not* in the gametes (eggs and sperm)
- Cannot be “selected” for in breeding programs



# **Genetic but non-inherited traits include:**

- **Developmental damage due to X-rays of fetus**
- **Teratogenic compounds**
- **Skin melanoma induced by UV irradiation**
- **Chemically induced leukemia**

# Why do statistical research?


- To determine if a trait is genetically controlled
  - Not genetic
  - Genetic but not inherited (“somatic”)
  - **Genetic and inherited**





# Inherited genetic traits are:

- Regulated by genes
- The altered gene/DNA is within the (gamete) egg and sperm
- ***Can be*** “selected” for in breeding programs



# Determine Heritability

Heritability estimates provide an indication of **HOW** much a trait is governed by genetics:

All genetic or all environment?

Mostly genetic input?

Mostly environmental input?

A little of both?



# To Determine Heritability

- We collect health data from affected dogs with associated pedigrees
- Health data and pedigrees correlated by Gibbs sampling and Bayesian statistical analyses
- Provides estimate of heritability



# Latest Information for Bearded Collies

- Health data on
  - 1470 dogs** (836 female 633 male, 1 not reported):
    - 92 Addisonian\*** (60 female\*\* 32 male)
    - 1261 Unaffected** (712 female 548 male)
    - 117 Unknown**

\* Addisonian as determined by Veterinarian administered ACTH stimulation test

\*\*Note: females are 57% of submissions

Also, prevalence cannot be determined from these data


Final note: when I last spoke we had samples from 266 Beardies



# Latest Information for Bearded Collies

- Hypothyroid Dogs reported: 37
- Addisonian Dogs reported to also be hypothyroid: 5
- Submissions ~ 25/month





# Heritability of Addison's Calculated

- Heritability in the Bearded Collie: 0.71
- Quite high indicative of strong genetic basis
- High heritability often associated with Mendelian Inheritance
- Similar to our other findings
  - 0.75 Standard Poodle
  - 0.73 Leonberger,
  - 0.76 Portuguese Water Dog

# Assess Mode of Inheritance

- Sometimes pedigree clearly demonstrates trait is governed by single gene
- Sometimes pedigrees are less obvious





# Single Gene Regulated

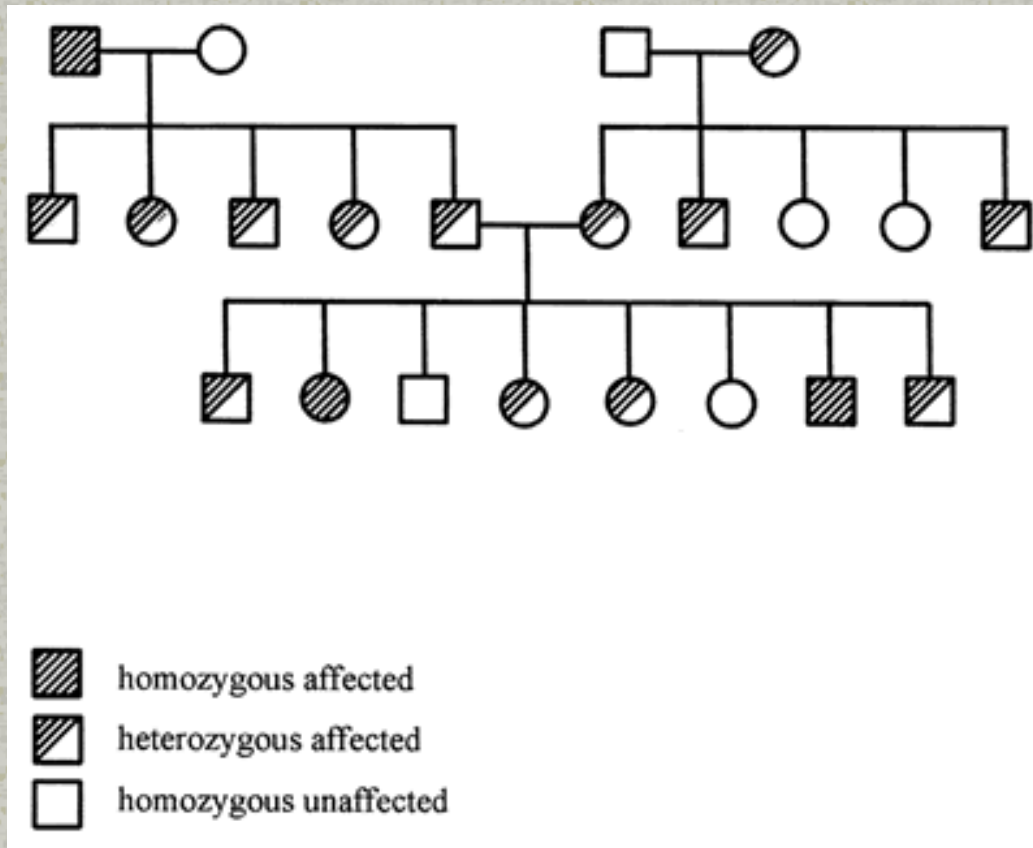




# Autosomal Dominant

- Males and females affected equally
- Regulating gene is **NOT** on the sex chromosomes (gene is on one of the 38 pairs of autosomes)
- At least one parent of affected offspring is also affected
- Trait does *not* skip generations

# Autosomal Dominant



Example: short hair coat



# Autosomal Recessive

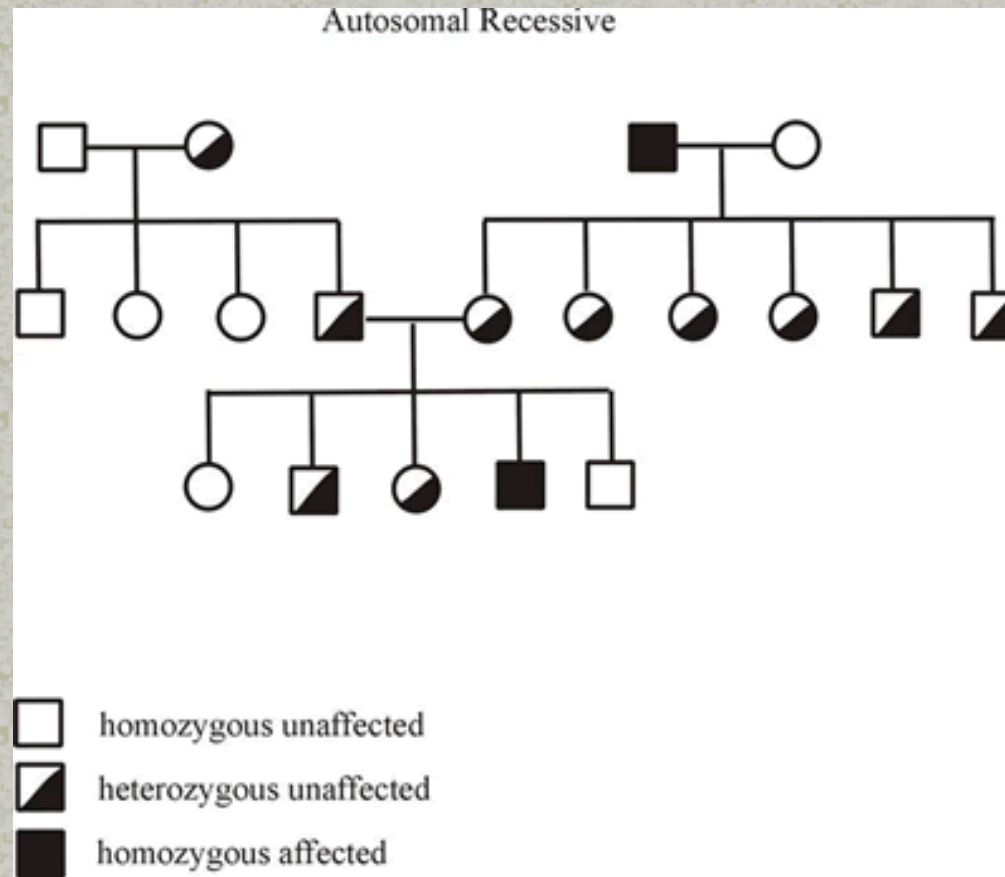
- Male and females affected equally meaning regulating gene is **NOT** on the sex chromosomes (gene is on one of the 38 pairs of autosomes)
- Affected dogs must be homozygous for the mutant allele



## **Autosomal Recessive (cont.)**

- **Each parent of an affected puppy must be a heterozygous carrier of the mutant allele**
- **A carrier bred to a carrier will produce on average 25% affected, 50% carriers, 25% normal**

# Autosomal Recessive




**Example: cerebellar ataxia**



# What do you do with this genetic information?

- For diseases:
  - If highly heritable and autosomal dominant, don't breed affecteds (for late onset disorders, develop genetic test)
  - If highly heritable and autosomal recessive, develop genetic test
- For traits:
  - Fix alleles that produce that trait



# Diagnostic genetic tests for breeding tools

DNA



Localize the disease gene to a physical location on one of the chromosome



Identify gene involved



Determine DNA change that alters protein



# DNA diagnostic tools

- **DNA test stock prior to breeding**
  - *Examples of available tests:*
    - Coat color tests for Labradors
    - PRA tests for numerous breeds
    - Congenital stationary night blindness (Briards)
    - von Willebrand's clotting disorder (Dobies, Poodles, Scotties, etc.)



# If Only That Simple...

- Many times there's no clear pattern of trait transmission
- Or mode of inheritance reflects the influence of more than a single gene
- Traits may be governed by many genes: polygenic or multifactorial or “incomplete penetrance” or “autosomal dominant with modifiers”

# How is Addison's Inherited in the Beardie?

- We know it has high heritability
- High heritability is often Mendelian



Let's look at a pedigree





# Polygenic Inheritance

- Both dam and sire contribute *one or more* mutant alleles to affected offspring
- No predictable ratios in pedigrees because number of genes involved is not known
- Both sexes affected, but possibly in unequal numbers

# Many Genes Regulating





# **Complex Segregation Analysis**

- **Used to determine mode of inheritance**
- **Trait is considered a discrete trait**
- **Analysis requires a regressive logistic model to assess presence of major gene**
- **Statistically compare various models (no major gene, major gene, recessive major gene)**



# Complex Segregation Analysis Applied to the Bearded Collie Data

- **Statistical evidence suggests that a single gene of large effect on the expression of Addison's disease exists with a recessive mode of inheritance** ( $P < 0.06$ )

Note: incorrect diagnosis in even one dog can cause rejection of major gene model; inbreeding doesn't help either

- **However, the evaluation for false positives is also significant which suggests caution in interpretation; Not true for Poodle data**



# What does “Major Gene” mean?

- A major gene is defined as one whose contribution has a significantly large influence on the expression of a trait
- Or more, narrowly: one that is necessary and sufficient to result in expression of the disorder at some point



## Major Gene Detected...

- Treat as if single gene for diagnostic:



## No Major Gene Detected...

- Genetic diagnostic difficult, therefore statistically rank breeding stock on genetic merit (EBV's)



# Next Steps in Addison's Work

- Addison's is being studied in several breeds simultaneously
- All indicate high heritability and likelihood of major gene
- Different Approaches:
  - Poodles, PWD, and Nova Scotia Duck Tolling Retrievers: Candidate Gene Approach
  - Beardies and PWD: Homozygosity Screening



# Candidate Genes:

- Genes that induce Addison's in humans cause Addison's in addition to a suite of other maladies
- Candidates induce disruptions to immune function and lead to autoimmune effects:
  - cytotoxic T lymphocyte antigen 4 (CTLA-4) and CD28—affect T cell responsiveness and recognition of self
  - *AIRE* gene—affects T cell tolerance
  - MHC class I and II



# Candidate Approach

- Scanned 182 highly related Standard Poodles, 92 PWD and a single large Toller family with canine microsatellites flanking candidates
- No LOD score (likelihood that the marker is linked to the expression of the phenotype) in excess of 1.0



# Homozygosity Scanning

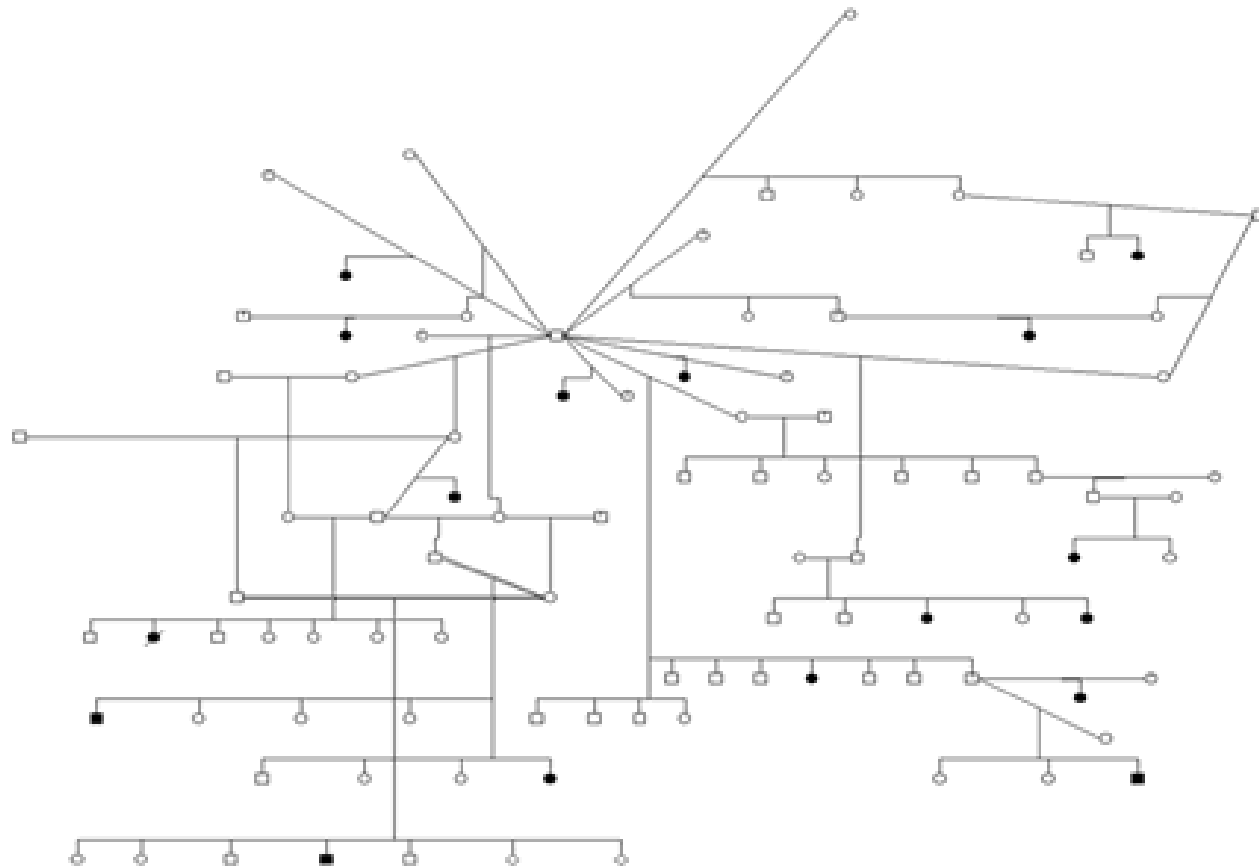
- Identified 8 Addisonian Beardies and 8 PWDs sharing no great grandparents
- Theory is that if they are unrelated, the only commonality between them is the DNA causing Addison's that they inherited from the ancestral "eve"



# Homozygosity cont.

- Scanned these dogs with 327 microsatellite markers that offer 9 Mb coverage
- Identified Chromosomes 1,9,11, 14, 17, 19, 20, 21, 23, 24, 25, 27, and 31 as targeted chromosomes to follow up
- Created a family of 96 Beardies, 17 reported as diagnosed Addisonian

# Beardie Family used




Note: using "ideal" autosomal recessive major gene, Lod score is  $> 6.0$

# Homozygosity Scanning

- All markers have been run on the sub family and linkage is currently being analyzed
- Goal is to find markers that co-segregate with the disease





# What can you do to help?

- Submit your dogs....all your dogs. We need accurate data on full families
- Update data on dogs you've submitted; even if healthy. Report ANY health aspect along with the DNA.
- Make sure the diagnoses are accurate; the results rely upon accuracy in reporting



# How to Participate

- **Simple easy request and collection procedure (buccal swabs can be requested at:**

**<http://cgap.ucdavis.edu>**

- **Anyone with a Bearded Collie can submit samples; we assemble the families at UC Davis**



# Birthday Party Swabbings





# What will this research do for Beardies?

- We've shown that Addison's is inherited
- Breeding advice: assume that Addison's is inherited as an autosomal recessive
- Scanning to locate causal chromosomal regions; goal is the development of genetic diagnostics
- Healthier dogs: remember, there's no shame in producing a problem if that is taken forward for the good of the breed.



# Collaborators

- As noted on title slide, Dr. Tom Famula
- Lab assistance: Janelle Belanger & Debbie Grossman
- Students: Kathy Benneman, Kristin Kellett, Kelly Regan, Ken Yagi
- Funding by AKC CHF & donations



**Thank you for your attention**

