Hereditary Diseases and Genetic Predispositions

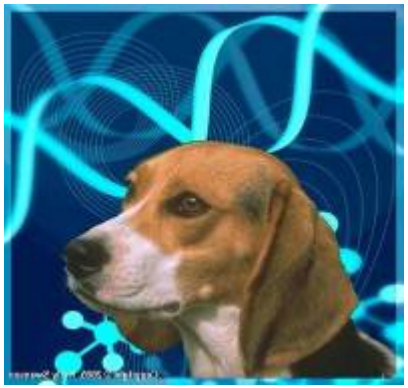
Urs Giger

PD Dr. med. vet. MS FVH

Dipl. ACVIM & ECVIM-CA (Internal Medicine)

Dipl. ECVCP (Clinical Pathology)

Section of Medical Genetics
School of Veterinary Medicine
University of Pennsylvania
Philadelphia



PennGen

Disclosures

- Scientific advisor to various companies including IDEXX, Purina, Royal Canin, and Waltham.
- Research support from various organizations including
  - National Institutes of Health
  - Canine Health Foundation
  - Winn Feline Foundation
- Director of the Genetic Disease Testing Laboratory

PennGen

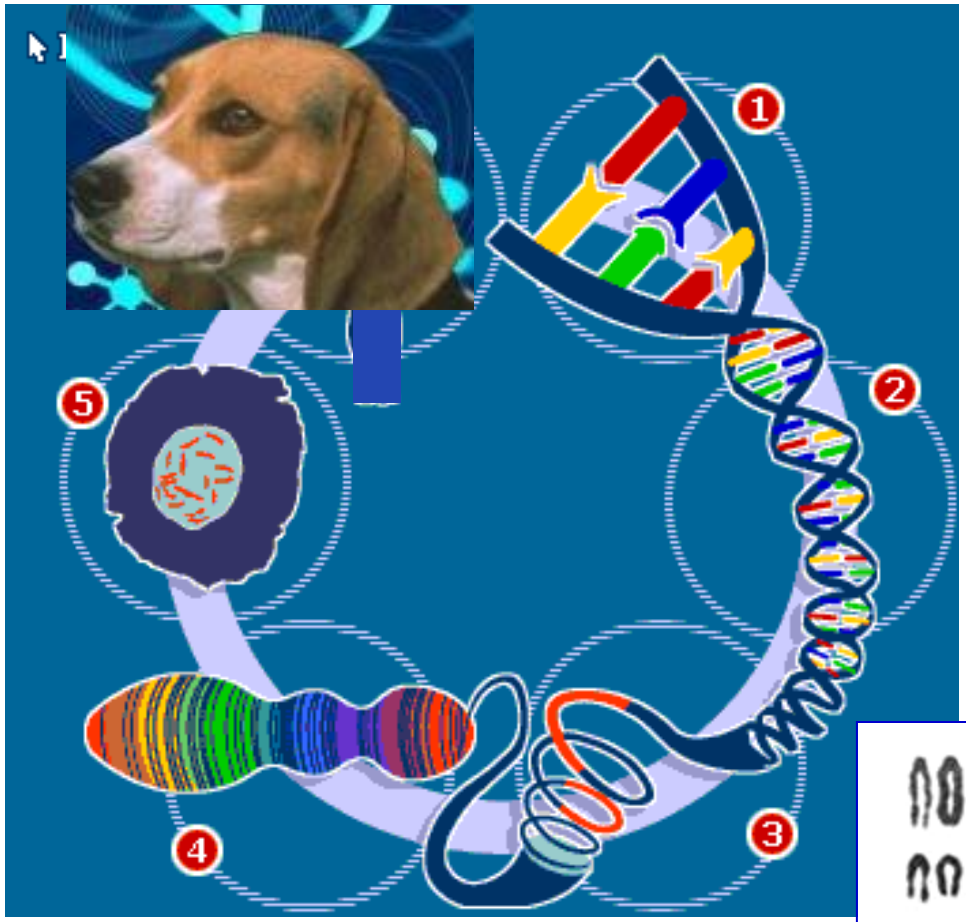
Acknowledgements

- My co-investigators at Penn Vet
- Many collaborators worldwide
- Veterinarians in many different clinics
- Numerous pet owners and breeders

Large Breed variations: ~400 dog breeds

- 175 AKC registered breeds
- All breeds are closely related.
- Breeds are genetic isolates.
- Some are geographically localized.

- Selected on basis of morphological & behavioral characteristics.
- Many breeds have narrow gene pools; minimal genetic diversity.
- Thus, many diseases are breed specific.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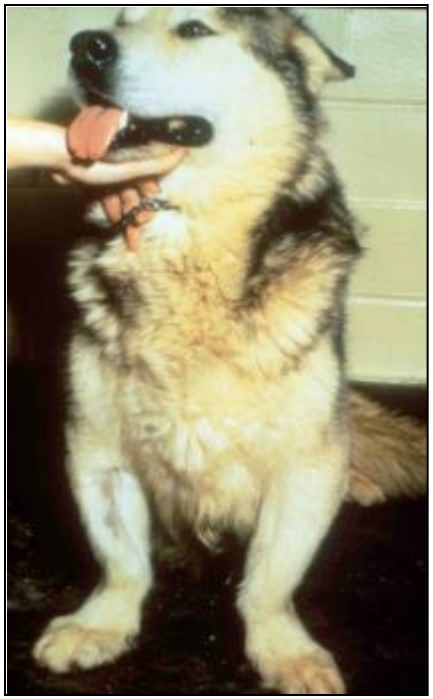
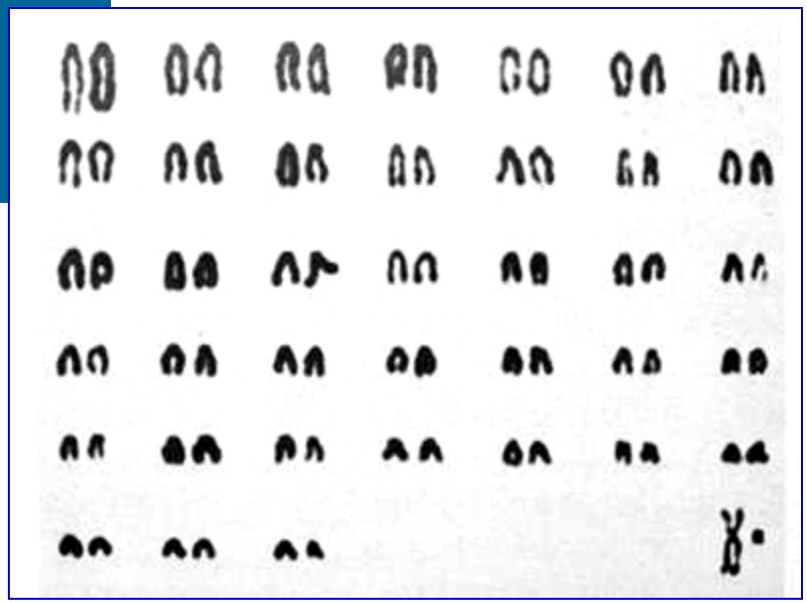
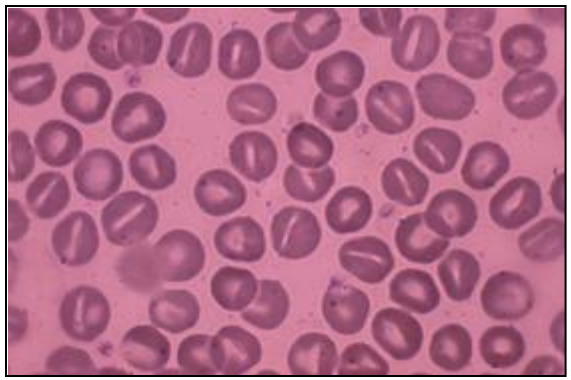
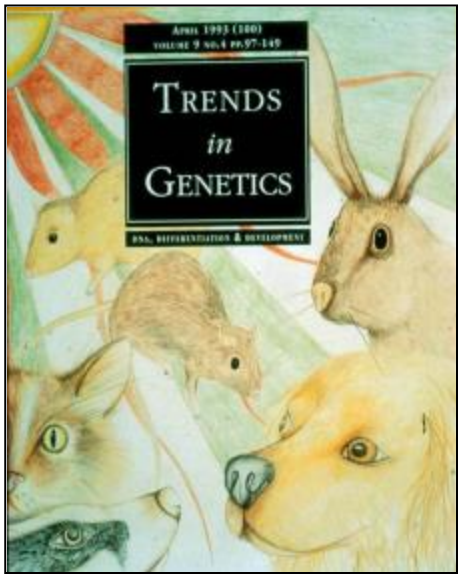






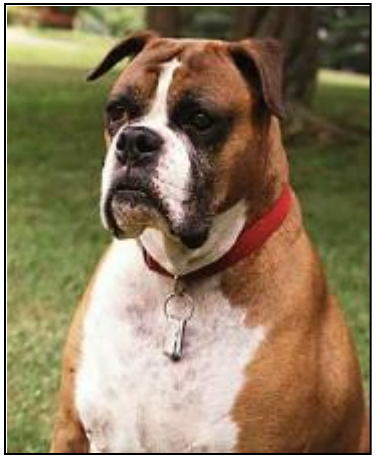
Sequence a dog (and cat) because…

- Dogs receive the highest degree of medical scrutiny beside humans.
- Dogs get essentially the same medical treatment as humans.
- Dogs serve as models for humans to evaluate novel treatment modalities.
- Dog/human DNA sequence similarity is higher than human/mouse.
- Dogs and humans share the same environments.
- Dogs exhibit greatest size and structural variation.
- Dogs are an example for evolution of carnivores and related species.

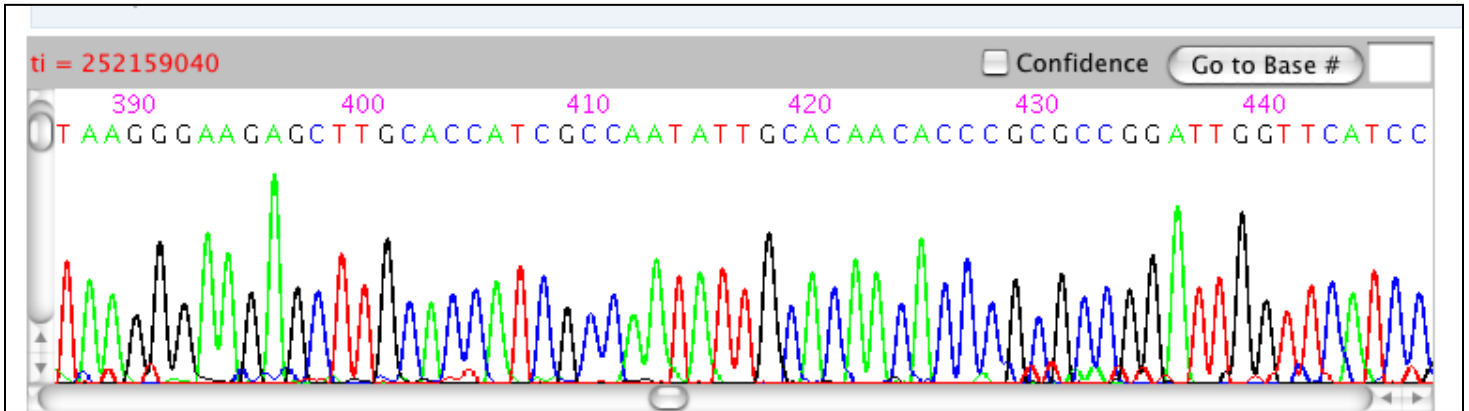


Canine Genome Sequence

achieved during past decade



- **First dogs sequenced** (US$100 millions)
  - **"Shadow" Poodle Celera TIGR Institute 2x**
  - **"Tasha" Boxer at MIT in Boston**
- **~3 billion bases**
- **~20,000 genes**
- **CanFam 3.1** Genome Sequence updated
- **Many more dogs sequenced ($3,000)**
- **SNP discovery in many breeds**
  - **Commercial microarrays (SNP chips)**



DNA Polymorphisms

A – Adenine G – Guanine

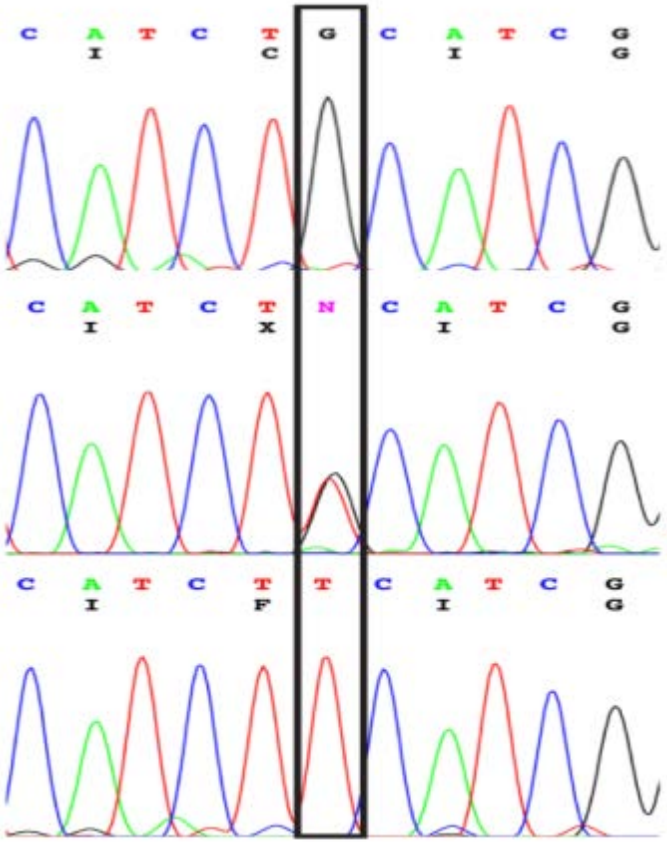
C – Cytosine T – Thymine

- Single Nucleotide Polymorphisms (SNPs)
- **Single base changes are called SNPs**
- **Some are variable between breeds and individuals of a breed.**
- **Currently used for Genome-Wide Association Studies (GWAS) to discover genetic traits.**
- **Also approach for complex traits**
- **Previously used microsatellites (repeats)**

SNP

CATCTGCATCG

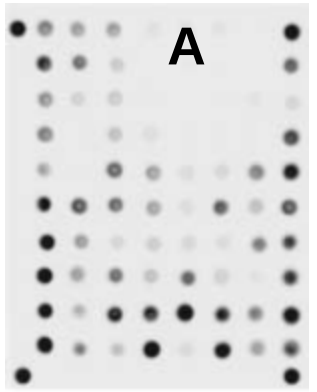
CATCTTCATCG

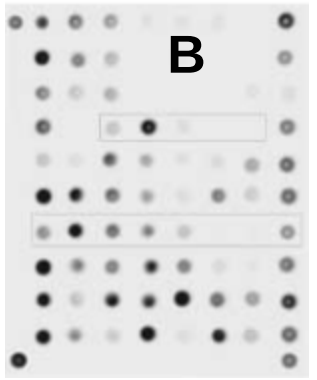


SNPs = Single Nucleotide Polymorphisms (170K)

On 38 canine chromosome pairs

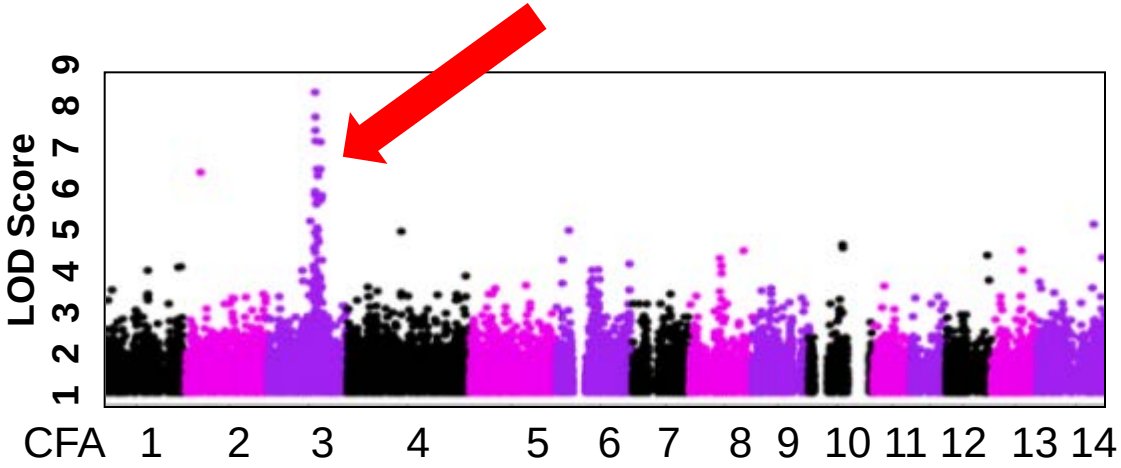
Green bars: Screening thousands of SNPs to look for variation

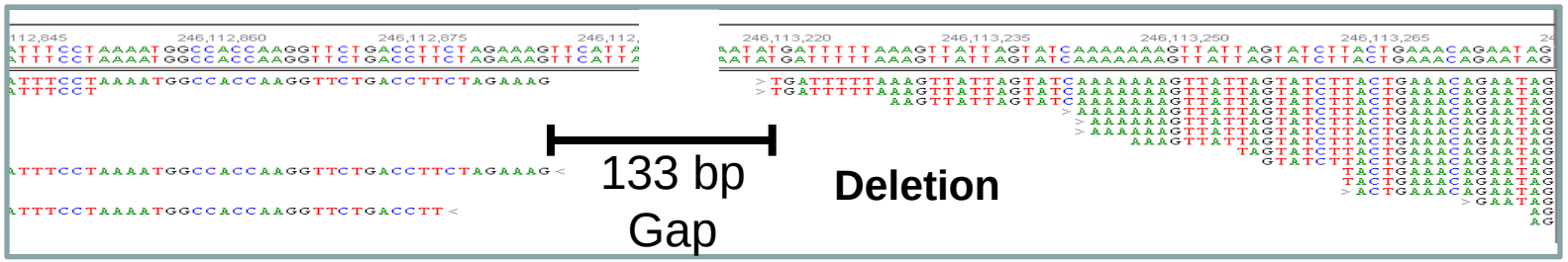




Canine Chromosomes (Autosomes)

Genomic Approach to Finding a Trait





Clinical Status	Genotype - Deletion			Total
	del/del	del/wt	wt/wt	
Affected	32	0	0	32
Unaffected	0	942	1778	2720
Total	32	942	1778	2752

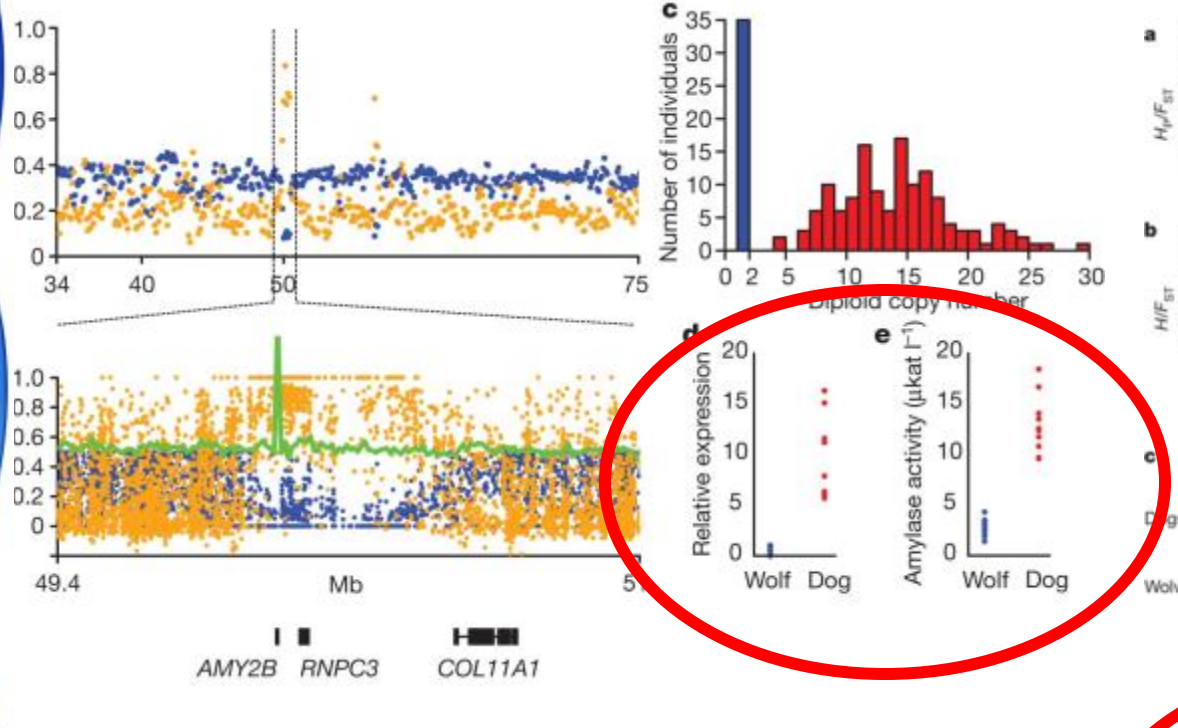
Effect on protein and metabolism needs to be determined.

Approach to Genetic Traits

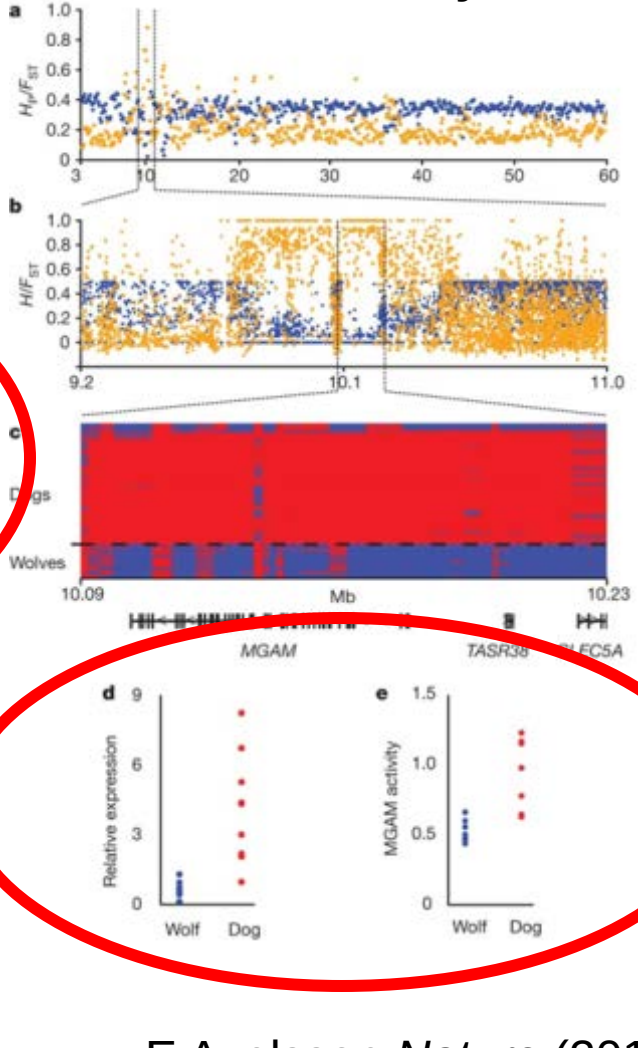
- **Candidate Gene(s)**
  - Based upon trait
  - Based upon comparison
  - Still many unknown genes and functions
- **Genomics**
  - Genome-wide association studies (GWAS)
  - Fine mapping
- **Whole genome sequencing**
  - Exomic sequencing
- *Biochemical, metabolic, hematological and clinic studies will be again needed.*

Canine Domestication from Wolf

Selection for increased amylase activity



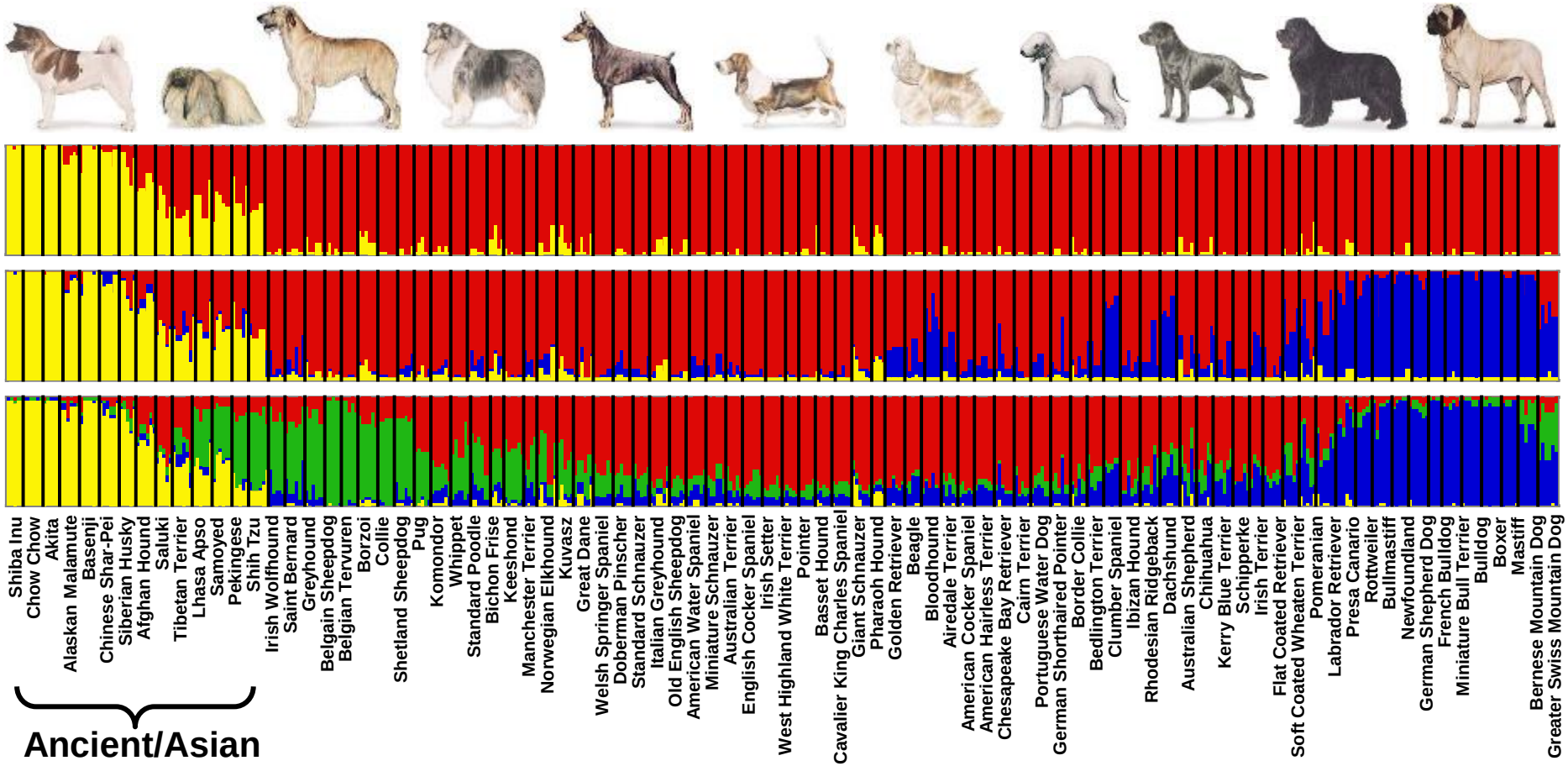
Selection for increased maltase activity



- Domestication from wolf
- Middle East ~14000 year
- Adaptation to starch metabolism
  - Amylase and maltase

E Axelsson *Nature* (2013)

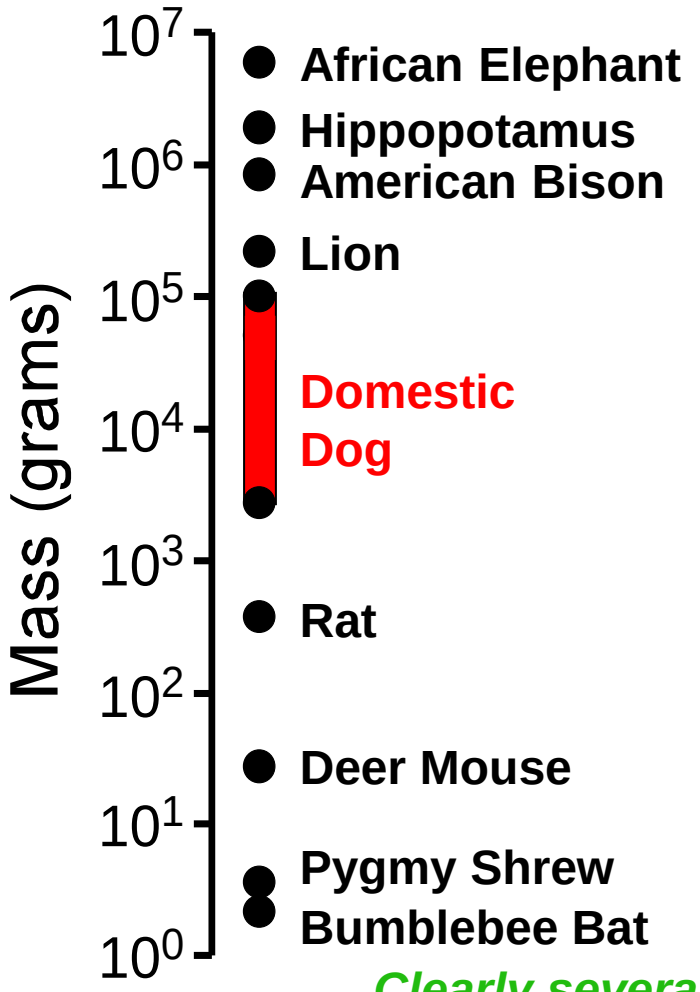
Population Structure of Dog Breeds

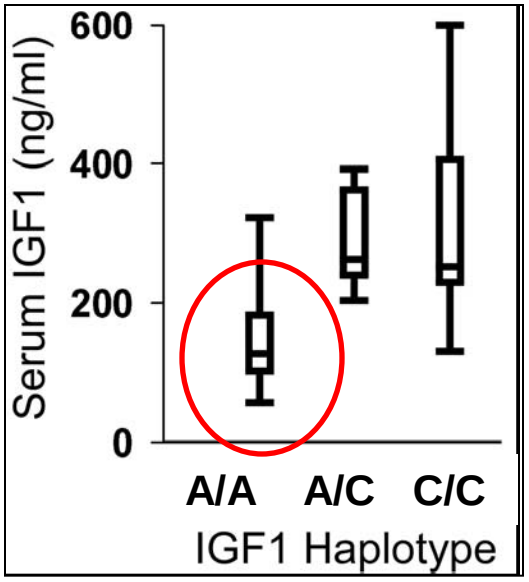


Ostrander, 2007 & 2012

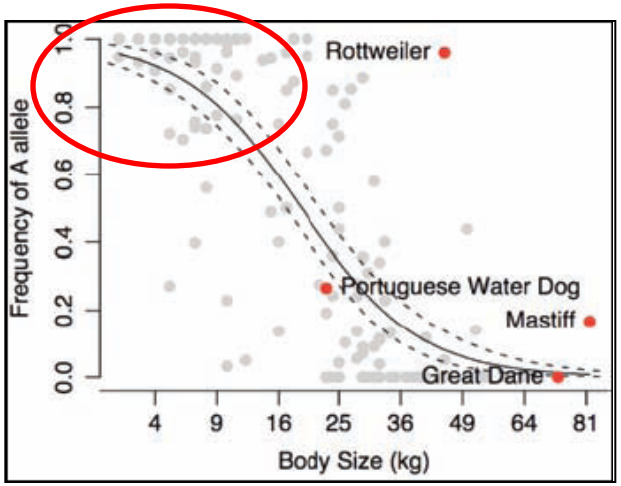
IGF1 ALLELE IS A MAJOR DETERMINANT OF SMALL SIZE

by genomics and candidate gene approach





Suter et al, 2007

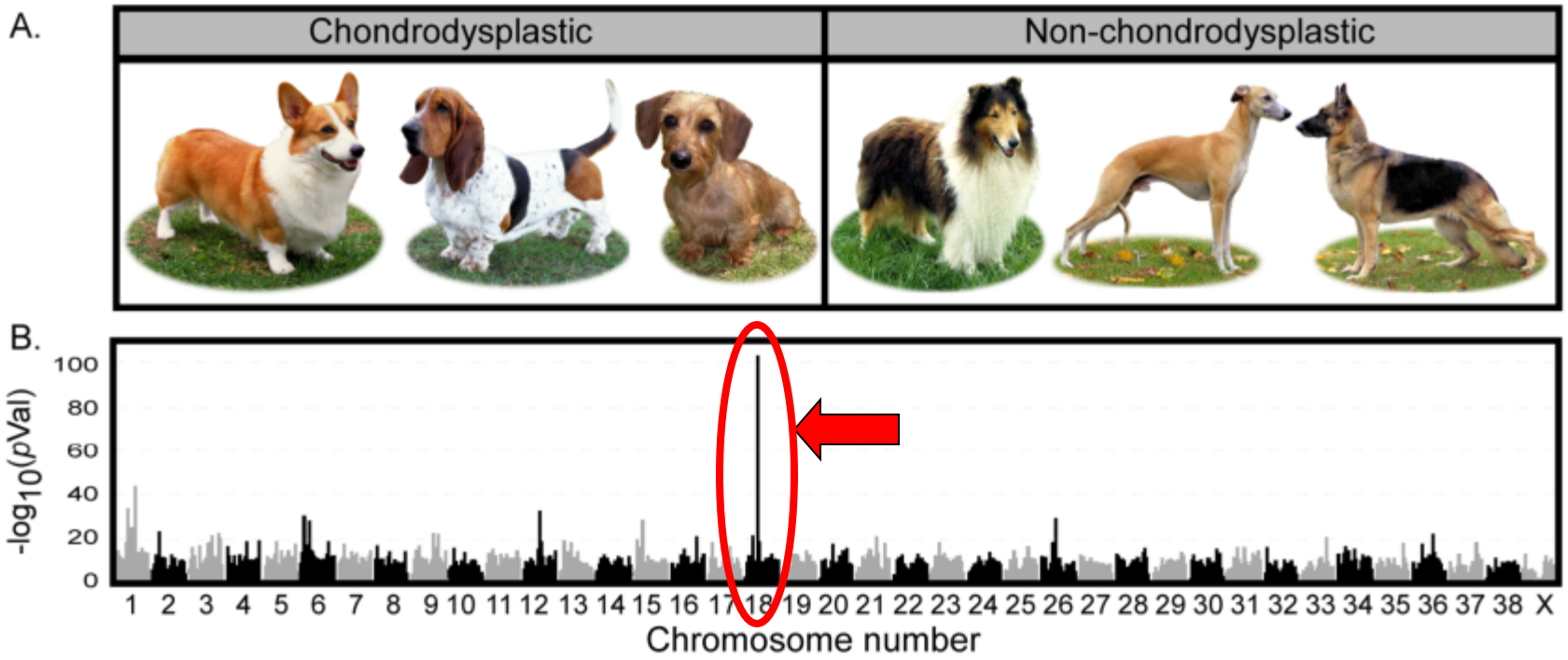


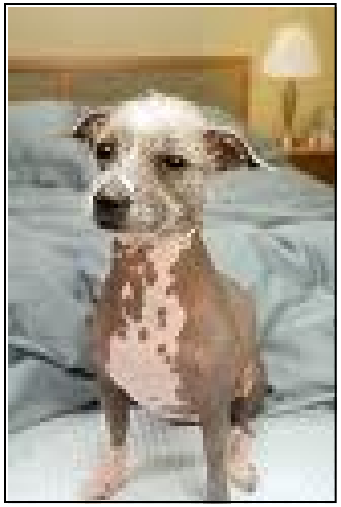
Clearly several other genes and factors are involved

Fibroblast Growth Factor Fgf4 Mutation is associated with Chondrodysplasia

- Several (19 AKC) breeds are short-legged
- High or low IGF-1 levels do not explain difference
- 40,000 SNPs, sequencing, breed association studies
- Retrogene of fibroblast growth factor 4 (fgf4)
- Gene insertion with abnormal functioning FGF4
- Abnormal leg growth regulation

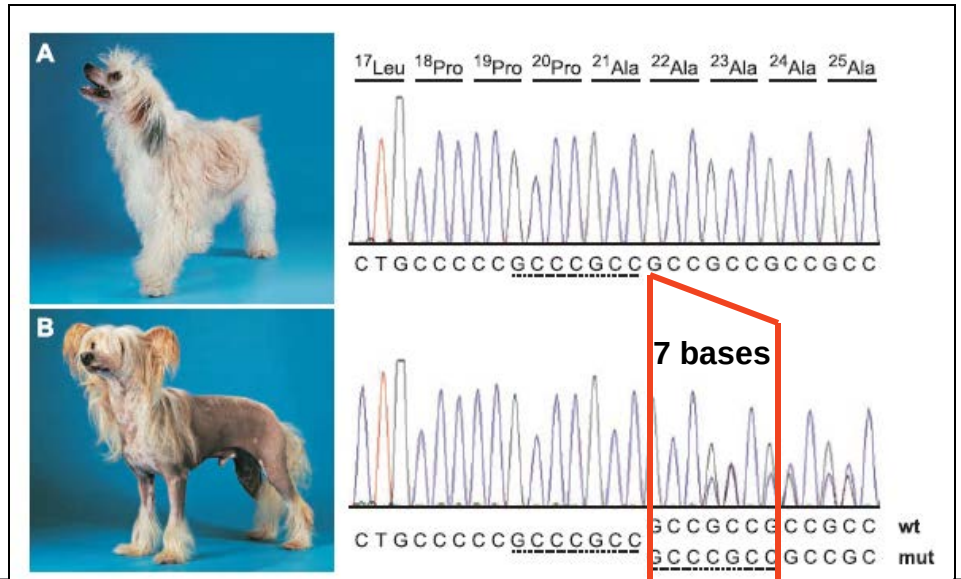
Parker et al, 2009





Hairless dogs (Ectodermal dysplasia)

- Known for >3700 years
- sacred by the Aztecs
- Missing or abnormally shaped teeth in addition to a sparse or absent hair coat
- Autosomal semidominant - homozygous lethal
- 7 base duplication in exon 1 of **forkhead box transcription factor 3** (FOXI3) causing frameshift & stop

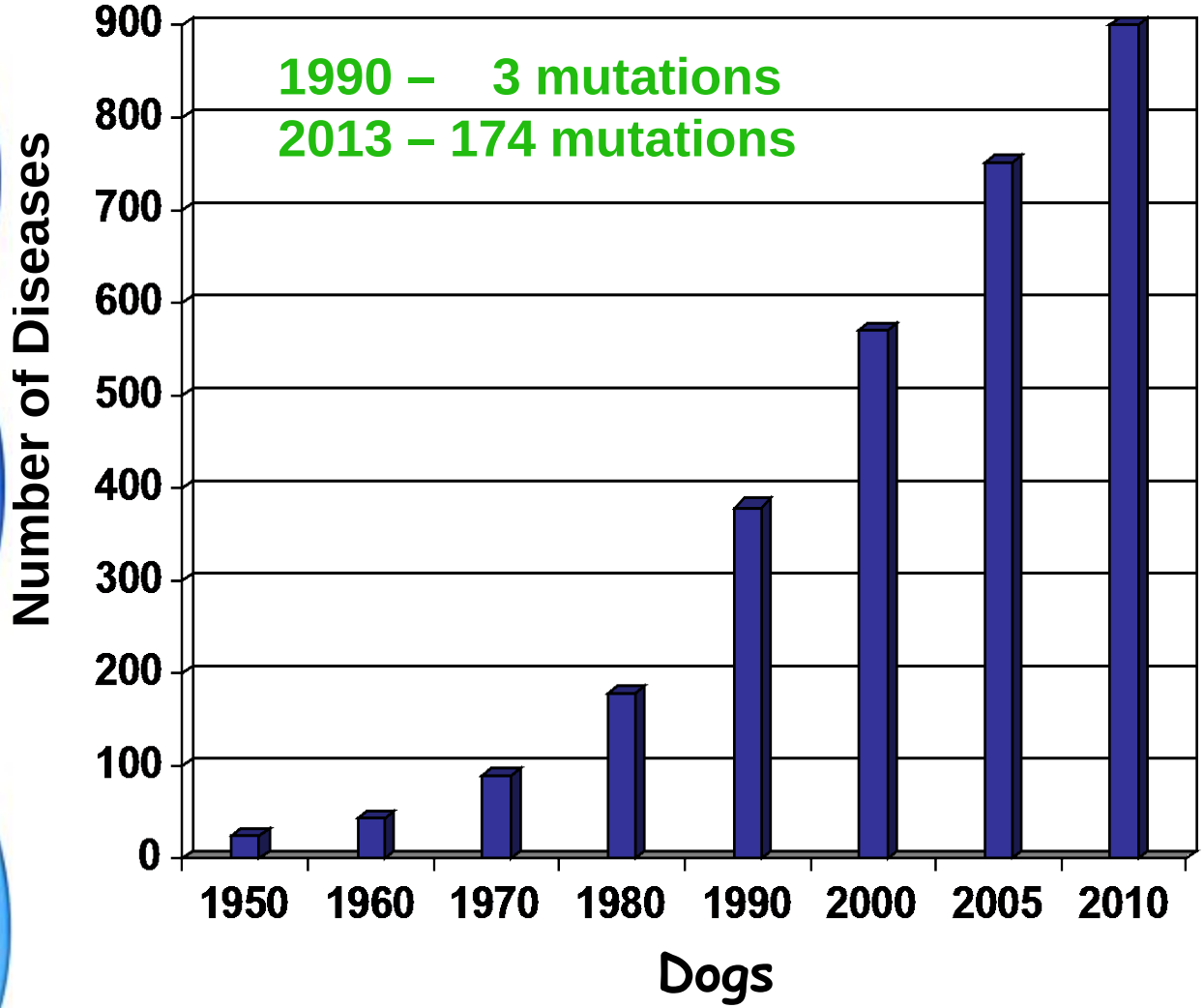


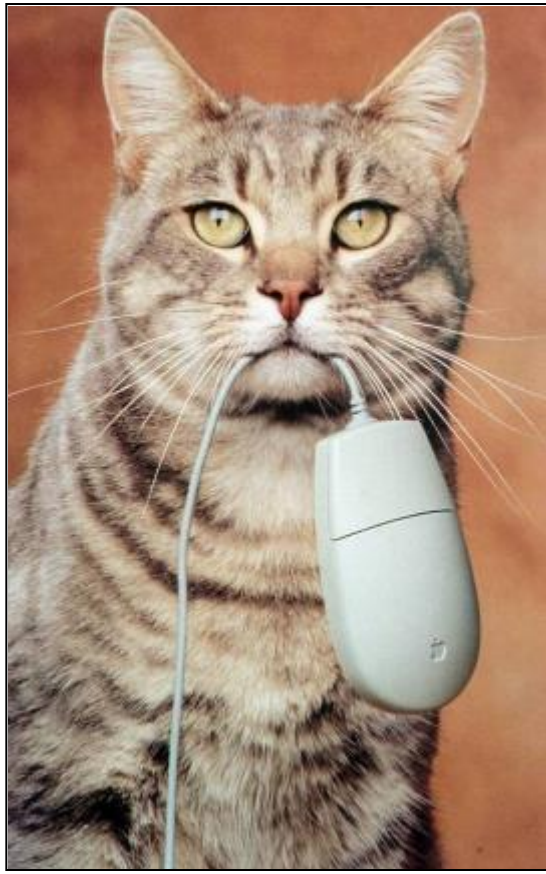
Drogenmueller, 2008

Cleo – What breed(s)?

1. Cannot be determined
2. Australian shepherd
3. Likely Dalmatian and Keeshond
4. Likely German shepherd and Labrador
5. Likely Husky and German shepherd
6. Other breeds

Hereditary Diseases in Dogs & Cats





~230 Hereditary Diseases in Cats

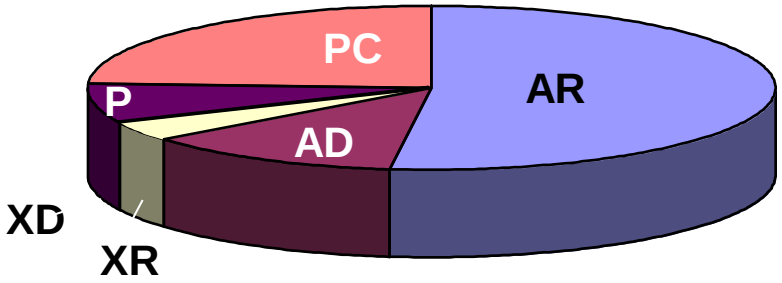
2013 – 24 mutations

"Inherited Diseases in Dogs" [IDID], http://www.vet.cam.ac.uk/idid

Mendelian Inheritance in Animals", http://www.angis.org.au/databases/BRIX/omia

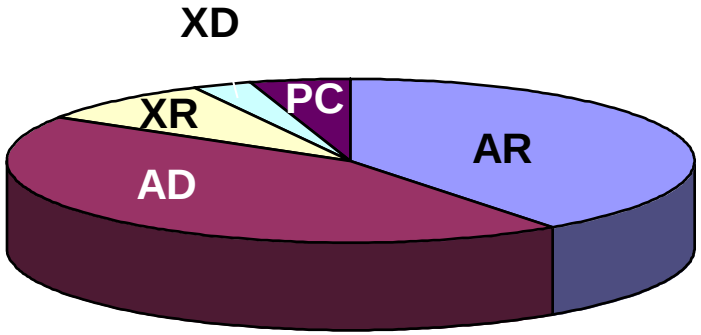
Modes of Disease Inheritance

- Most diseases in dogs and cats are inherited in an autosomal recessive trait or polygenic manner



Canine (IDID)

Inherited Diseases in Dogs
(http://www.upei.ca/~cidd/intro.htm)



Human (OMIM)

Online Mendelian Inheritance in Man

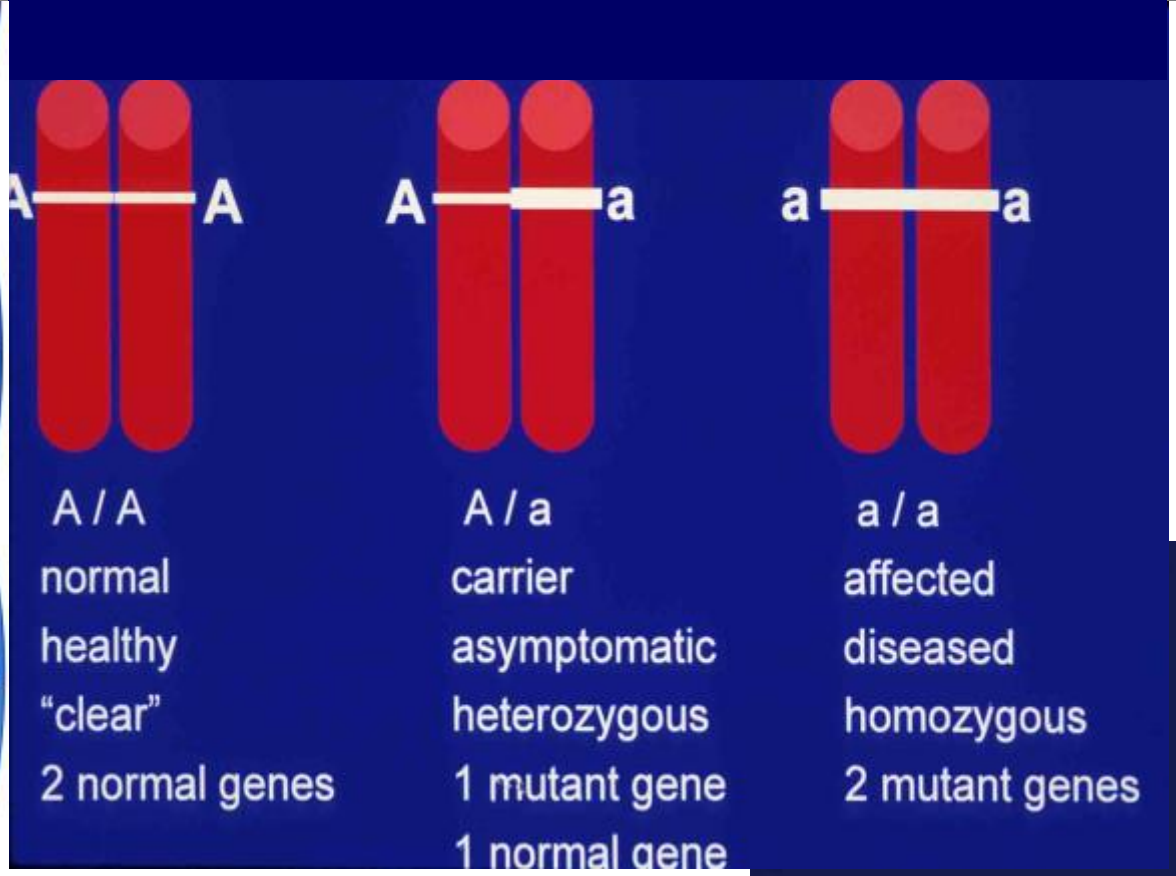
AR = autosomal recessive; AD = autosomal dominant;
XR = X-linked recessive; XD = X-chromosomal dominant;
PC = polygenic, complex, autosomal recessive.

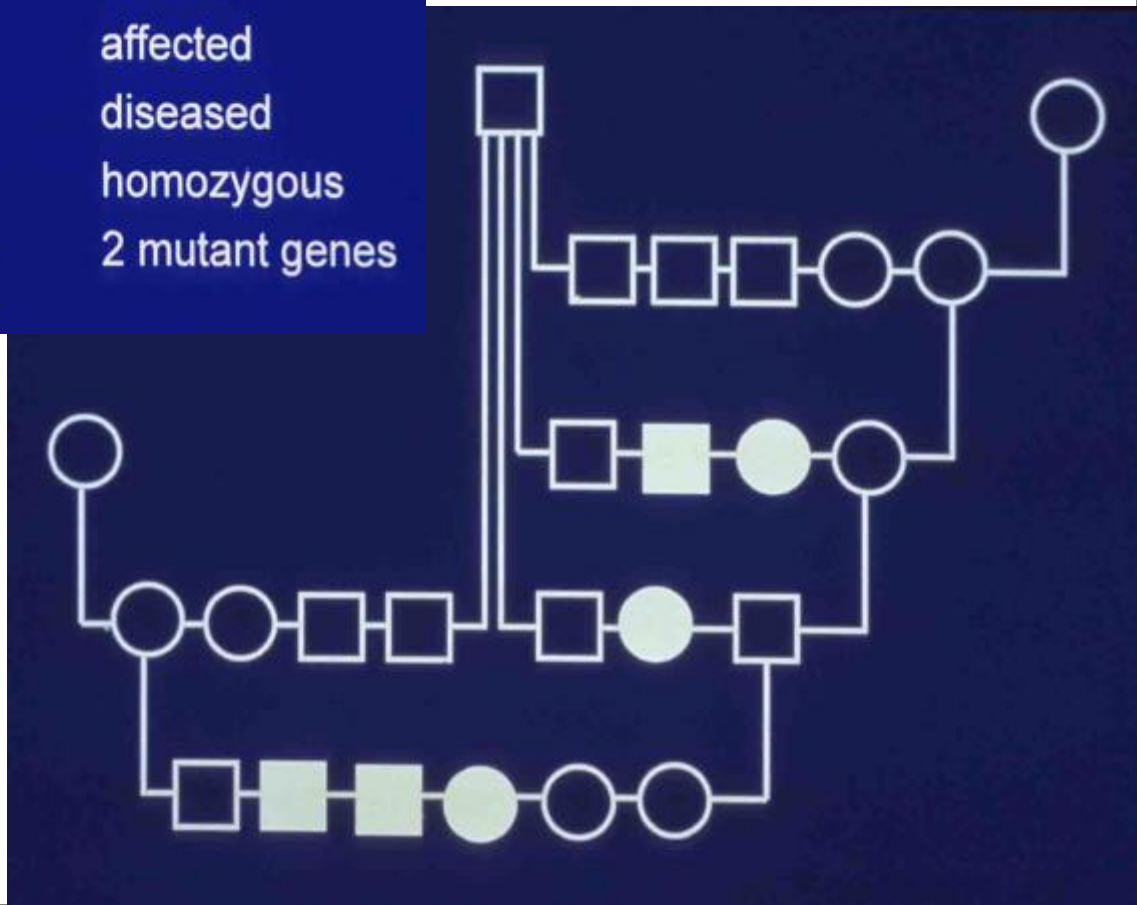
~2013

The Dog and Its Genome. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2006:249-289.

Autosomal Recessive (AR) Inheritance

- most common
- asymptomatic carriers
- "skips generations"





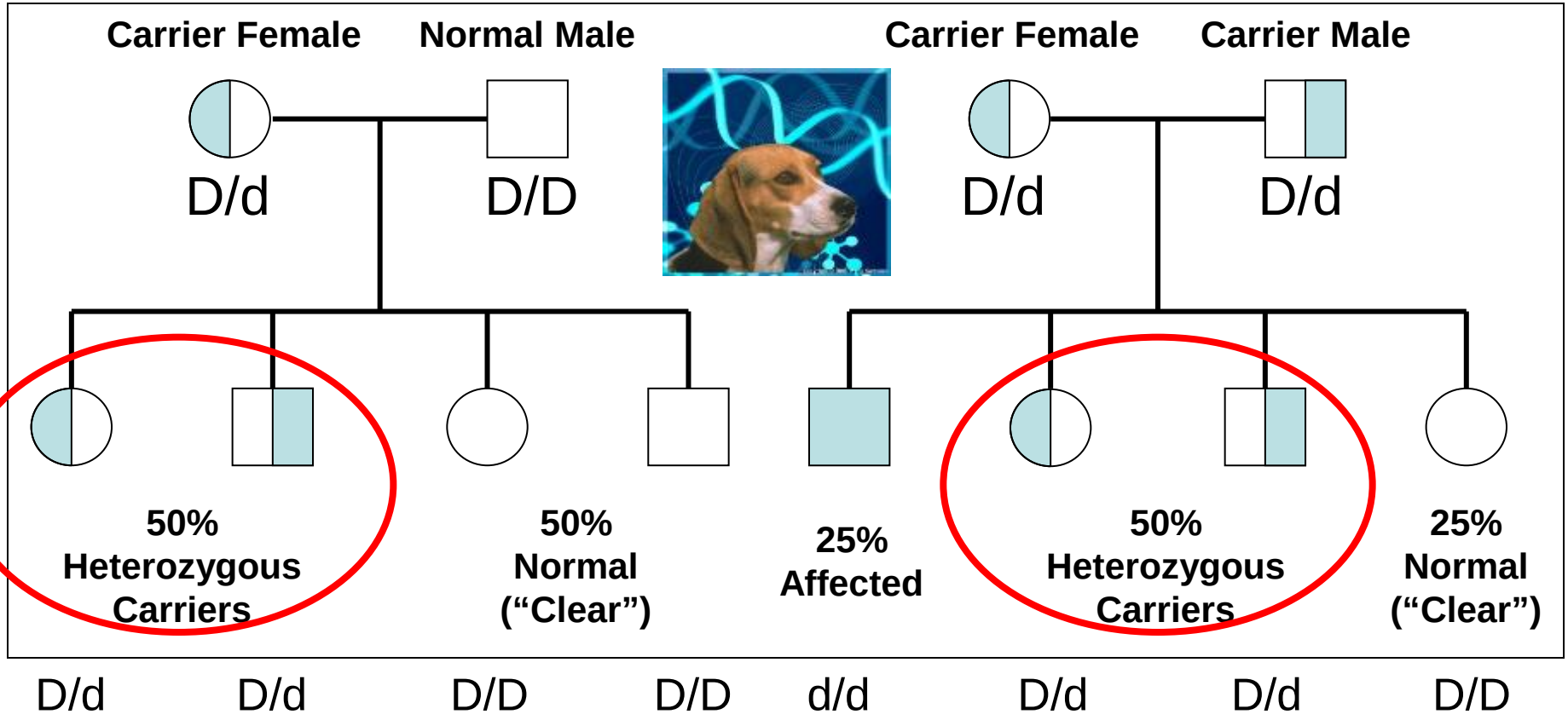
Males - squares

Females - circles

Affected – filled white

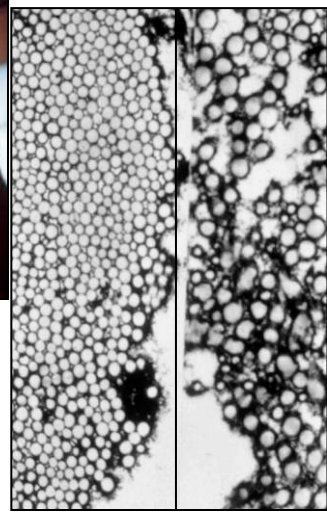
Soon complex traits more common

Autosomal Recessive Inheritance



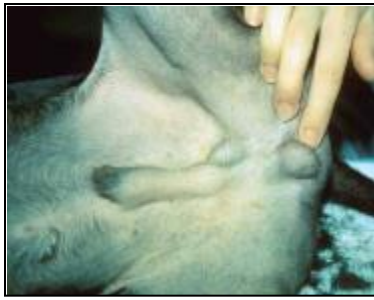
The majority of the mutant alleles underlying a recessively inherited disorder are spread in the population by heterozygous, clinically asymptomatic/ unaffected dogs.

Hereditary Diseases



Simple – single gene defects

- Autosomal recessive – dominant
- X-chromosomal recessive – dominant
- Still minor variation between affecteds



Complex – polygenic – genetic association

- Major and modifying genes, predisposition
- Environmental influences
- Large variation in time of onset and severity

Mitochondrial (very rare)

- Maternal transmission (mitochondrial DNA)
- Exertional myopathies, sensory ataxic neuropathy

Hereditary Diseases

Congenital malformations

- Developmental anomalies

Inborn errors of metabolism

- Enzyme, receptor, transporter defects

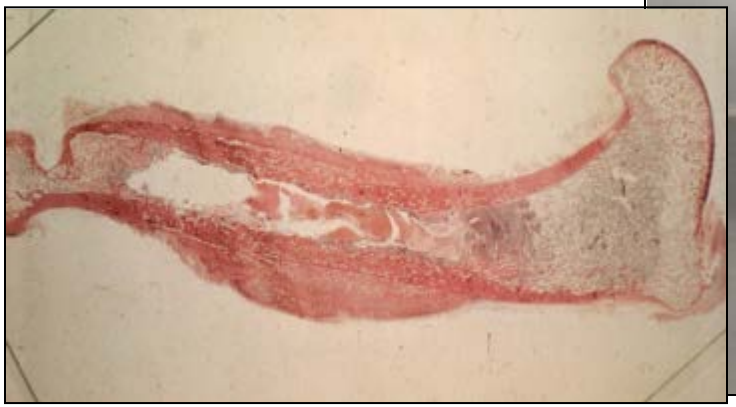
Genetic predispositions

- Infections
- Inflammations
- Immune diseases
- Degenerative processes
- Immunodeficiencies
- Behavioral disorders
- Pharmacogenetics
- Cancer

All 3 can overlap and likely have a metabolic basis

Genetic Disease Predispositions

- Infection
- Inflammation/immune-mediated
- Degeneration
- Behavior (aggression)
- Pharmacogenetics
- Cancer
- Others



Top 10 Canine Health Concerns
(AKC Canine Health Foundation)

#1 Hip Dysplasia

#2 Allergies

#3 Epilepsy

#4 Hemangiosarcoma

#5 Hypothyroidism

#6 Lymphoma

#7 Patella Luxation

#8 Cataracts

#9 Bloat

#10 Atopic Dermatitis

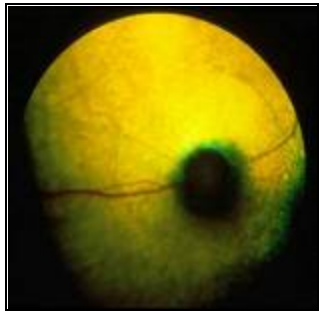
Others: Osteosarcoma, Autoimmune Disease, Renal Dysplasia, Portosystemic/hepatic Shunt, Elbow Dysplasia, Deafness, Progressive Retinal Atrophy

Still need a lot of research to define these complex traits.

Genetic Disease Testing

Methods

- Signalment (breed)
- Physical examinations
- Imaging (Rads, U/S, CT)
- Eye examination
- Routine laboratory tests
- Failing biological system analysis
  - Metabolites
- Protein assays
  - Quantity
  - Activity
- DNA analysis
  - Mutation tests
  - Linkage tests



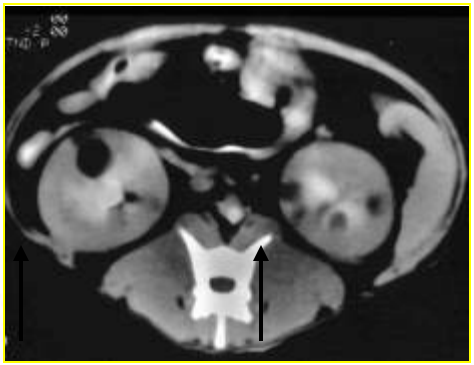
Phenotype

- **The observed clinical findings as determined by the genotype and the environment**
  - Carriers/heterozygotes of recessive disorders are asymptomatic

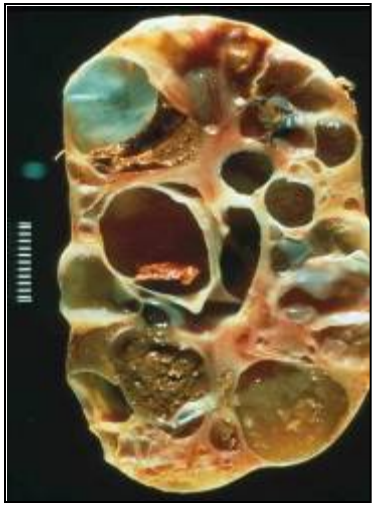
Genotype

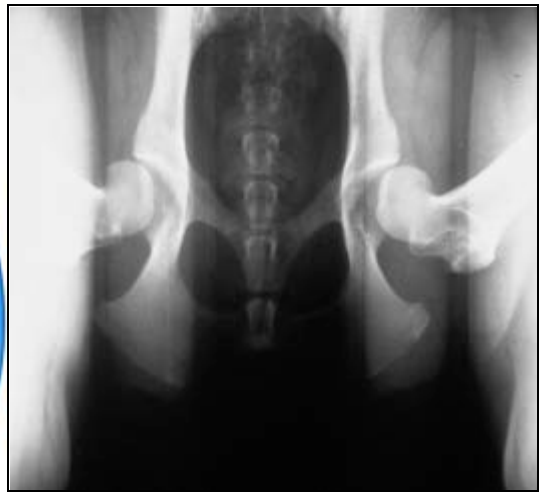
- **The genetic constitution or more specifically the alleles present at one gene locus**
  - Homozygous (affected)
  - Heterozygous (carrier)
  - Complex traits

Genetic Disease Testing:
Affected Animals

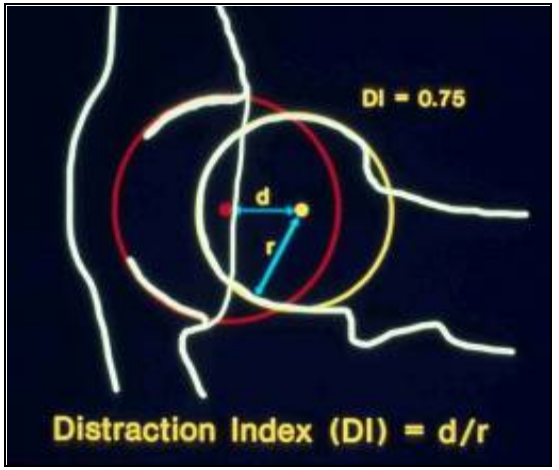


- Identify diseased animals
- Discover animals at risks
- Prior to developing signs
- Prior to selling into homes
- Prior to breeding
- Prior to training dogs





Physical examinations
Imaging (x-rays, U/S, CT)
Eye examination
Pathology
Laboratory tests
Failing biological systems
Protein assays
DNA tests



Passive hip joint laxity is a primary risk factor for the development of OA

Passive laxity

Weight bearing ↓

Functional laxity

↓

Excess stress on cartilagenous structures

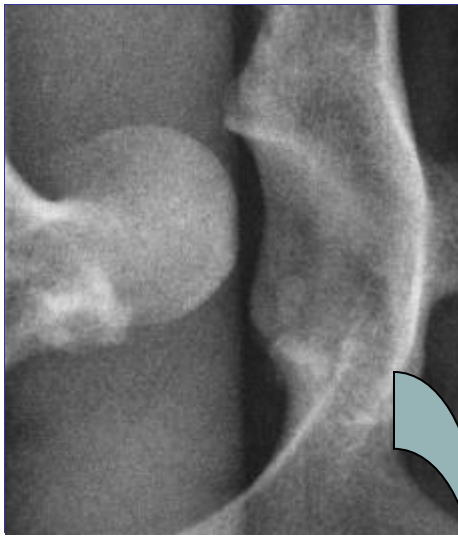
↓

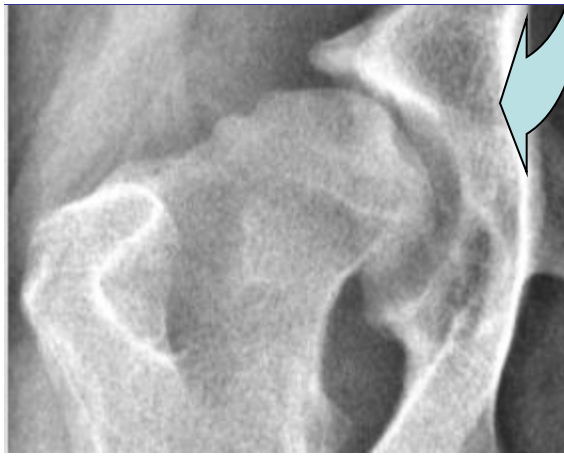
Microfractures, release of inflammatory mediators

↓

Periarticular osteophytosis, sub-chondral bone sclerosis, joint remodeling

Osteoarthritis

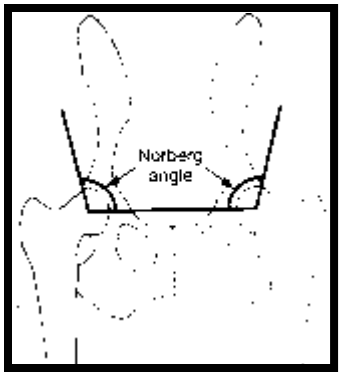




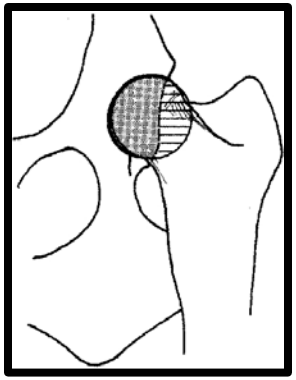
PennHIP

Hip Dysplasia & Laxity

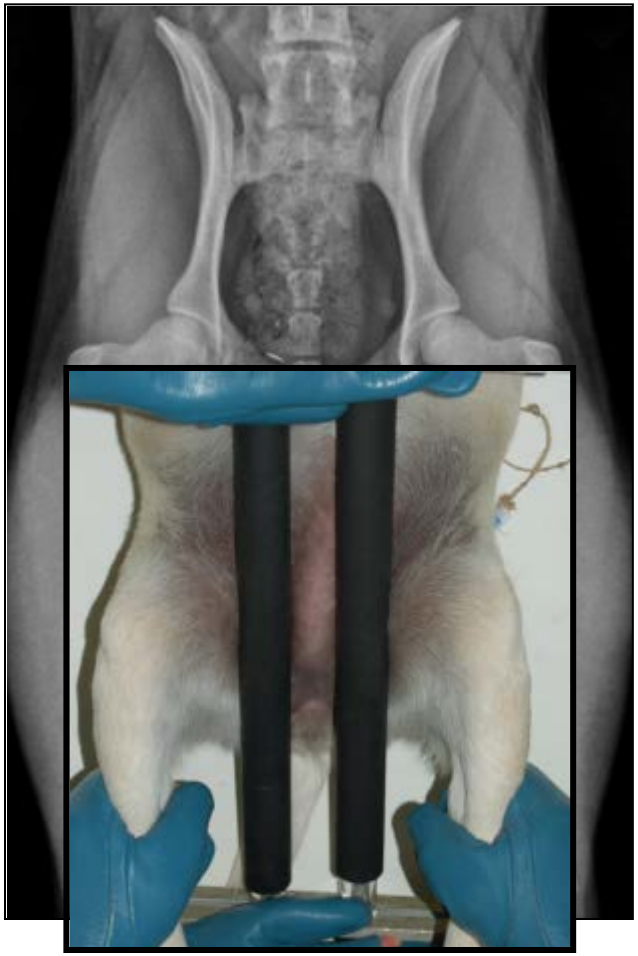
- Ventrodorsal, hip-extended radiographic view
- PennHIP views give best and earliest laxity results



Norberg Angle
≥ 105°

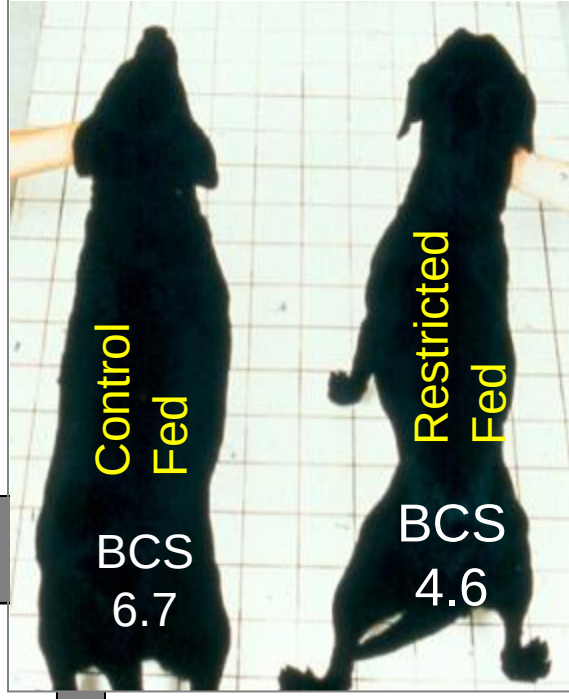


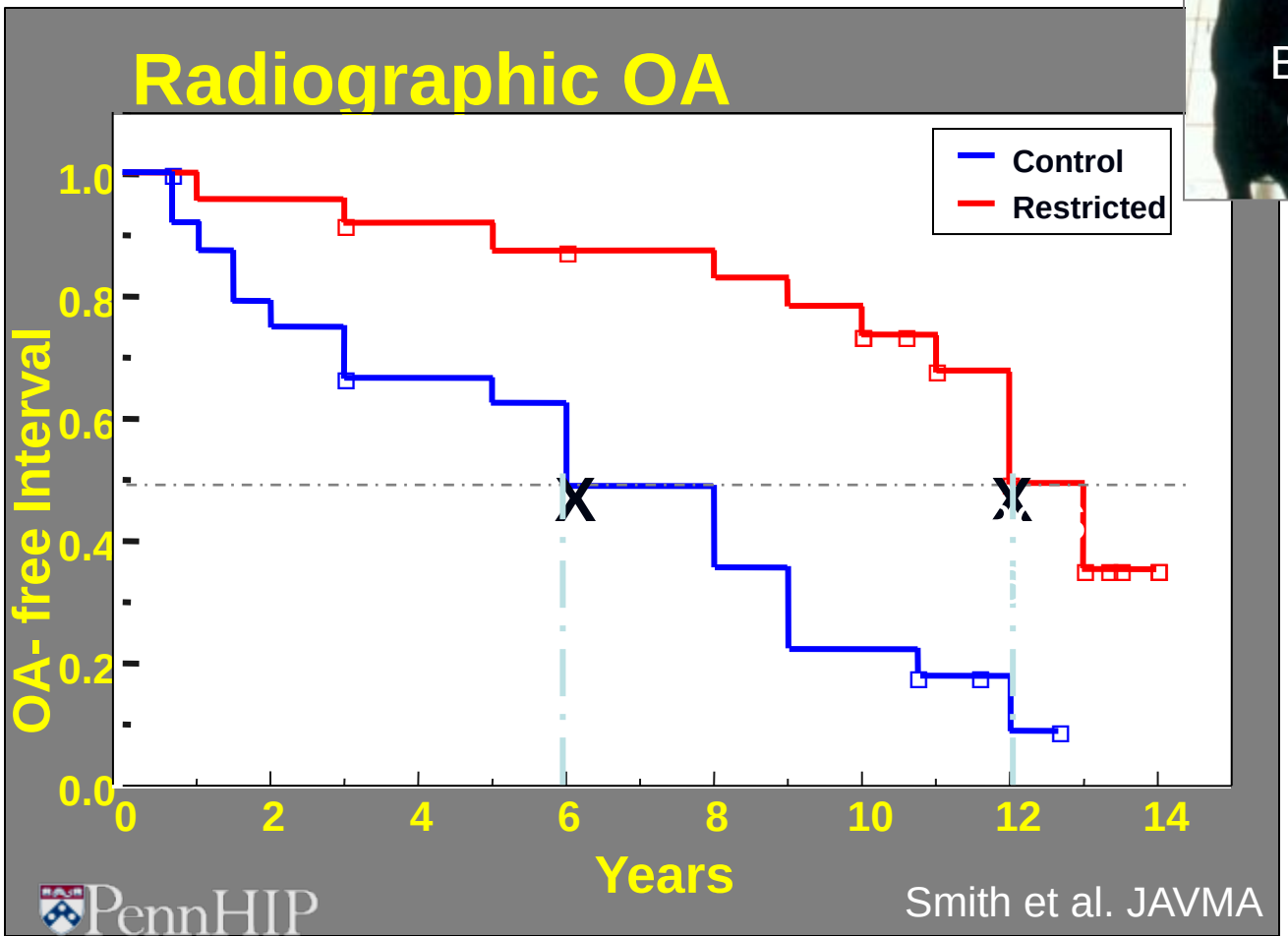
% Femoral Head
Coverage

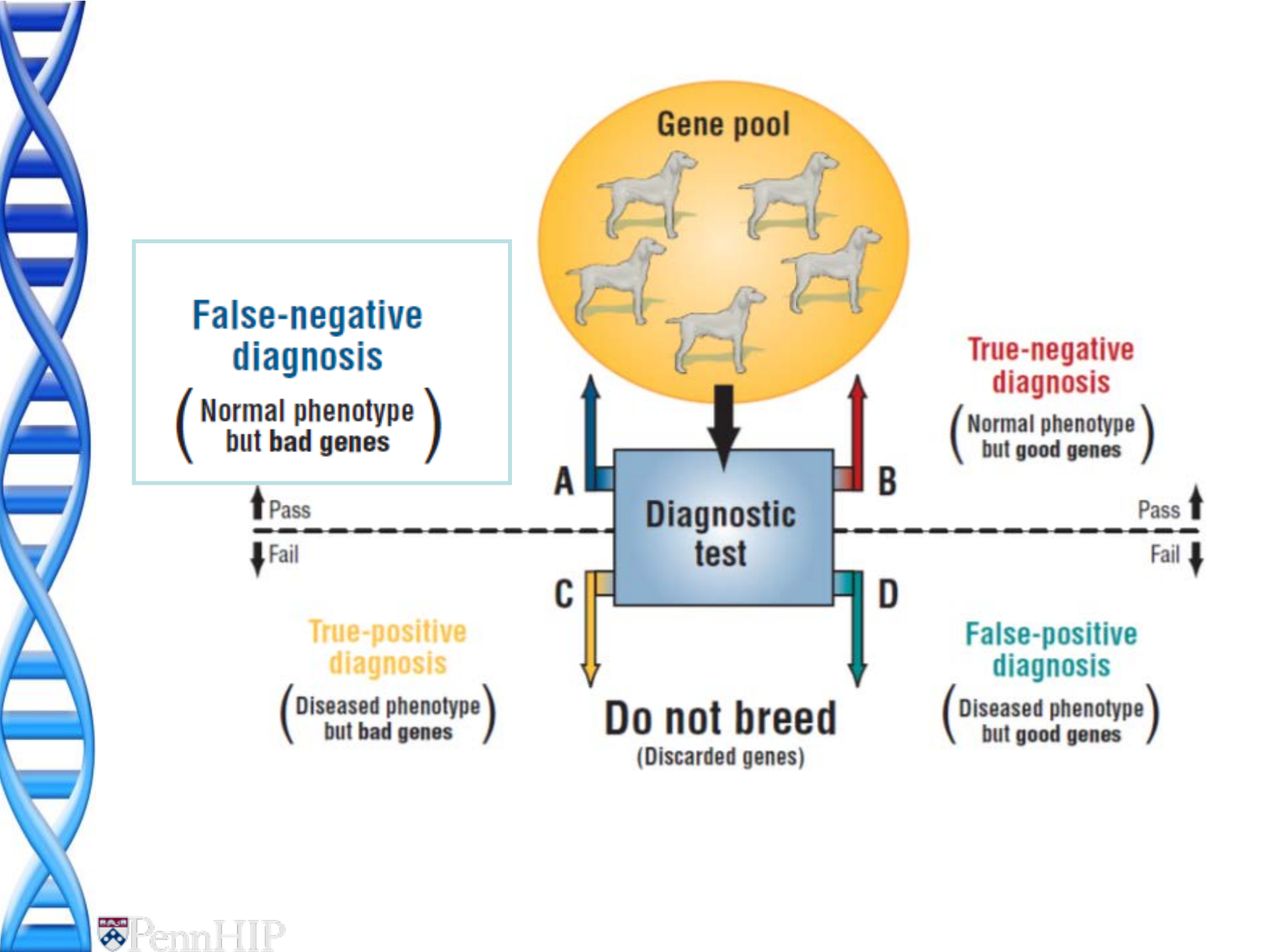


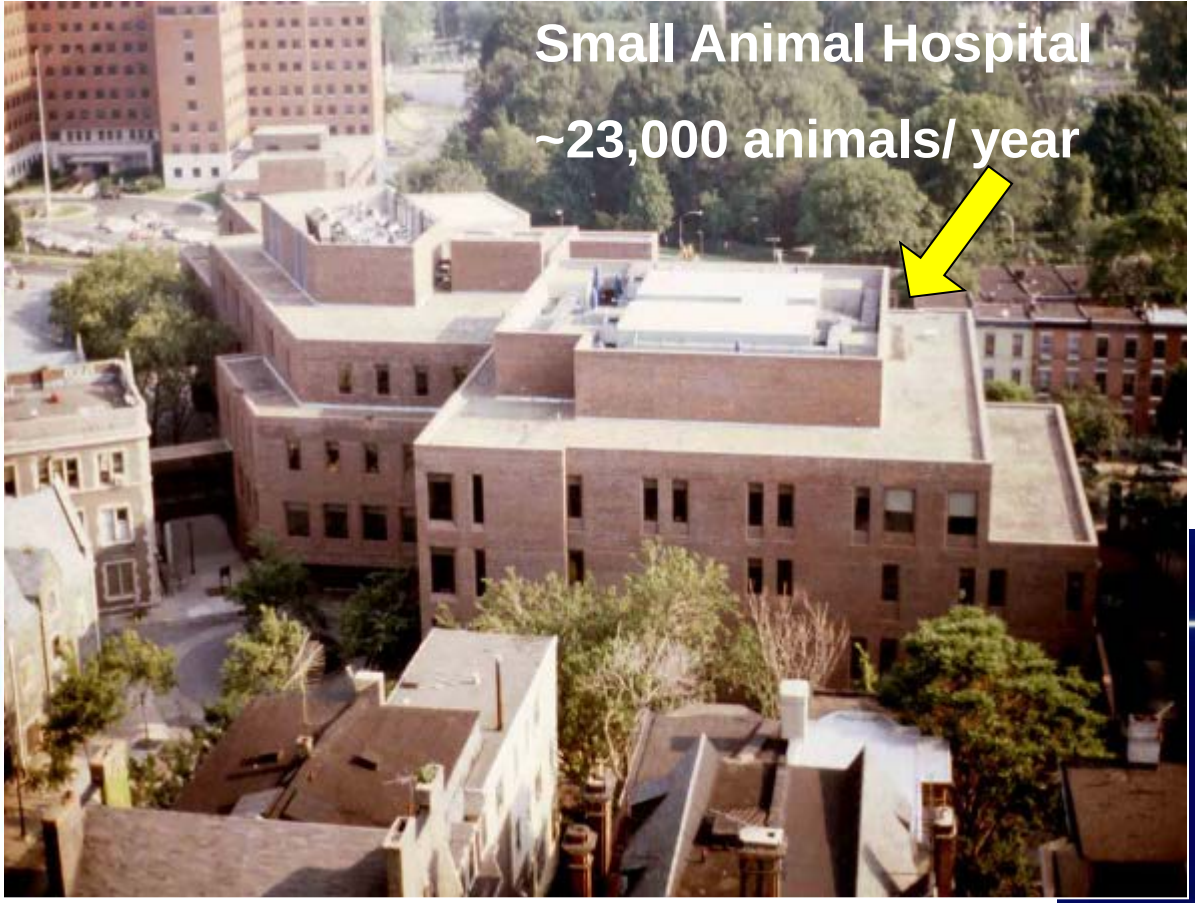
Smith & PennHIP

Osteoarthritis & Diet






Gene pool
False-negative diagnosis
(Normal phenotype but bad genes)
True-negative diagnosis
(Normal phenotype but good genes)
A
B
Pass
Fail
Diagnostic test
Pass
Fail
C
D
True-positive diagnosis
(Diseased phenotype but bad genes)
False-positive diagnosis
(Diseased phenotype but good genes)
Do not breed
(Discarded genes)

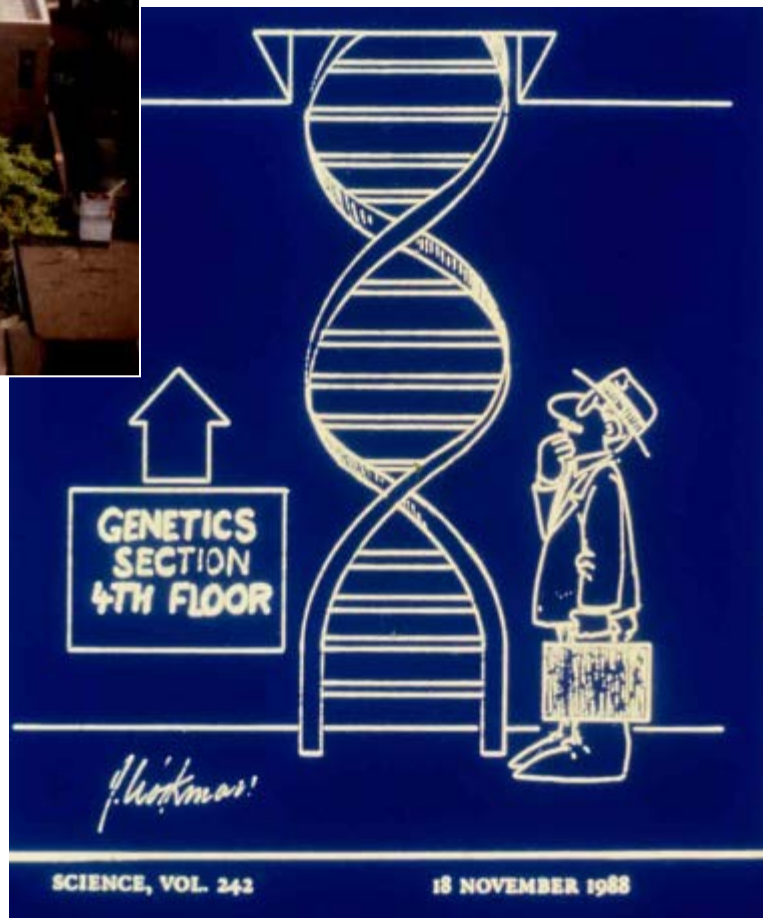


University of Pennsylvania

School of Veterinary Medicine

Section of Medical Genetics

- **Pediatrics and Genetics Clinic**
- **Metabolic Genetics Screening Laboratory**
- **Josephine Deubler Genetic Testing Lab**
- **Genetic Disease Research Groups**
- **Gene Therapy Research Group**

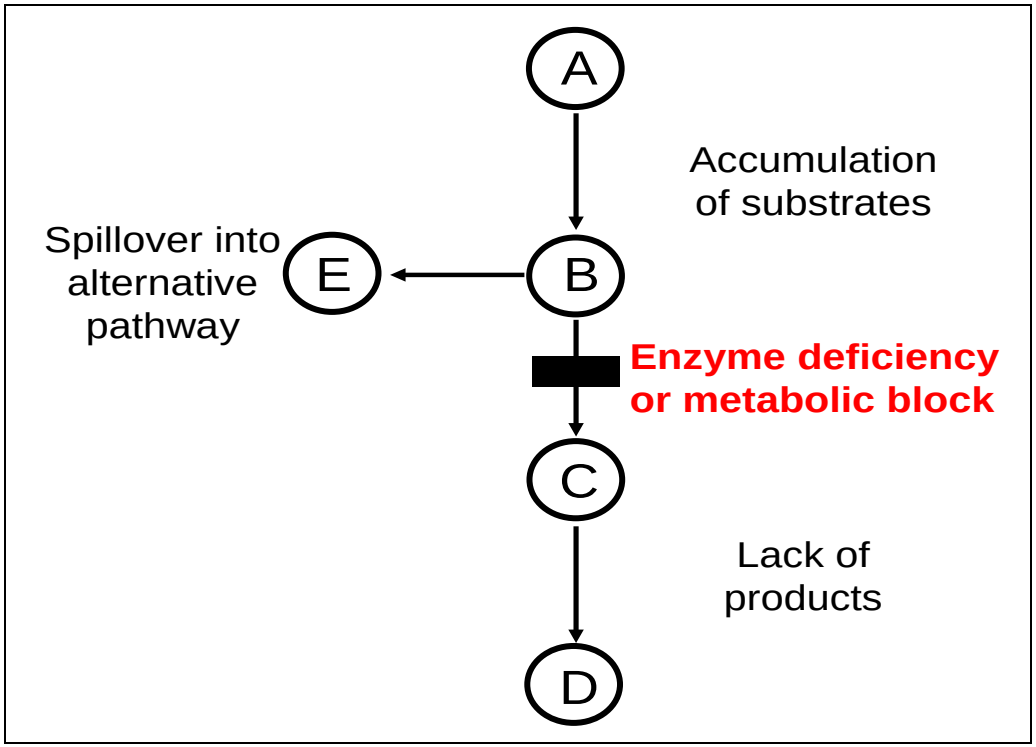


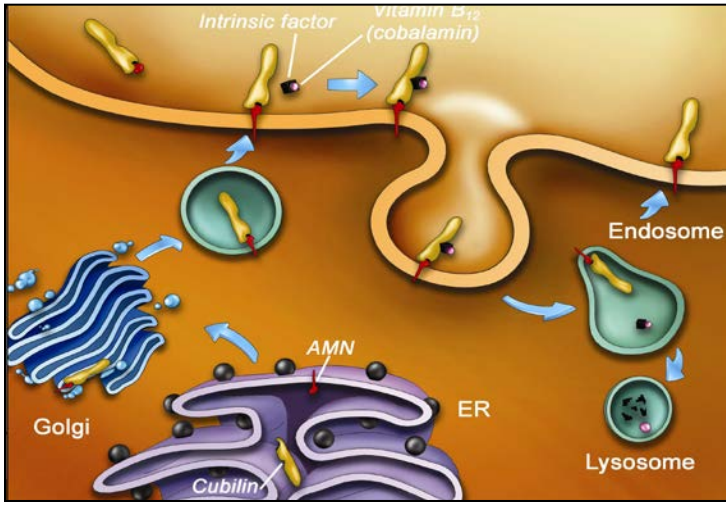
Inborn Errors of Metabolism

- Currently refers to single gene defects
- With the better characterization of hereditary disorders, practically all genetic defects could be considered to be an inborn error of metabolism including malformations and susceptibility to disease.

Sir Archibald Garrod 1910

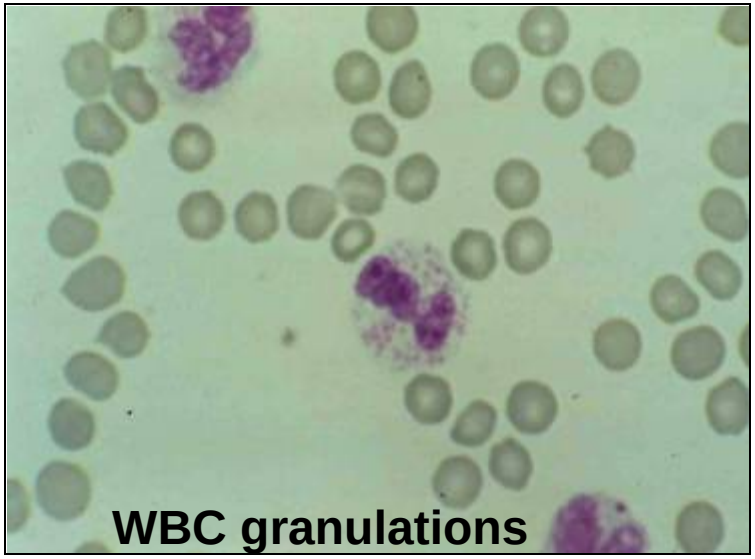
Metabolic consequences in a pathway

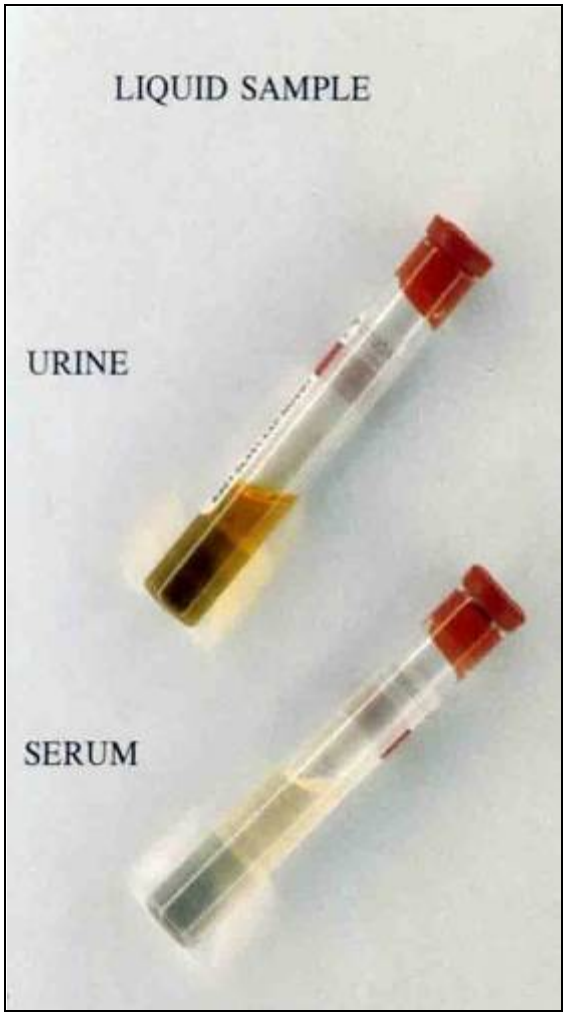




- Enzyme deficiencies
- Structural proteins
- Receptors, adhesion molecules, ion channels
- Plasma proteins

Metabolic Genetic Screening Tests





Severe lipemia

Metabolic Genetic Screening Tests

PennGen

Urine

Spot Tests

Paper Chromatography

MPS spot

Clinitest
Ketostix

Nitroprusside
test

Amino Acid

Organic Acid

\+

\+

\+

\+

\+

MPS
electrophoresis

Carbohydrate
chromotography

Amino acid analyzer

MS/Gas
chromatography

Chondroitin sulfate
Dermatan sulfate
Heparin sulfate
Keratan sulfate

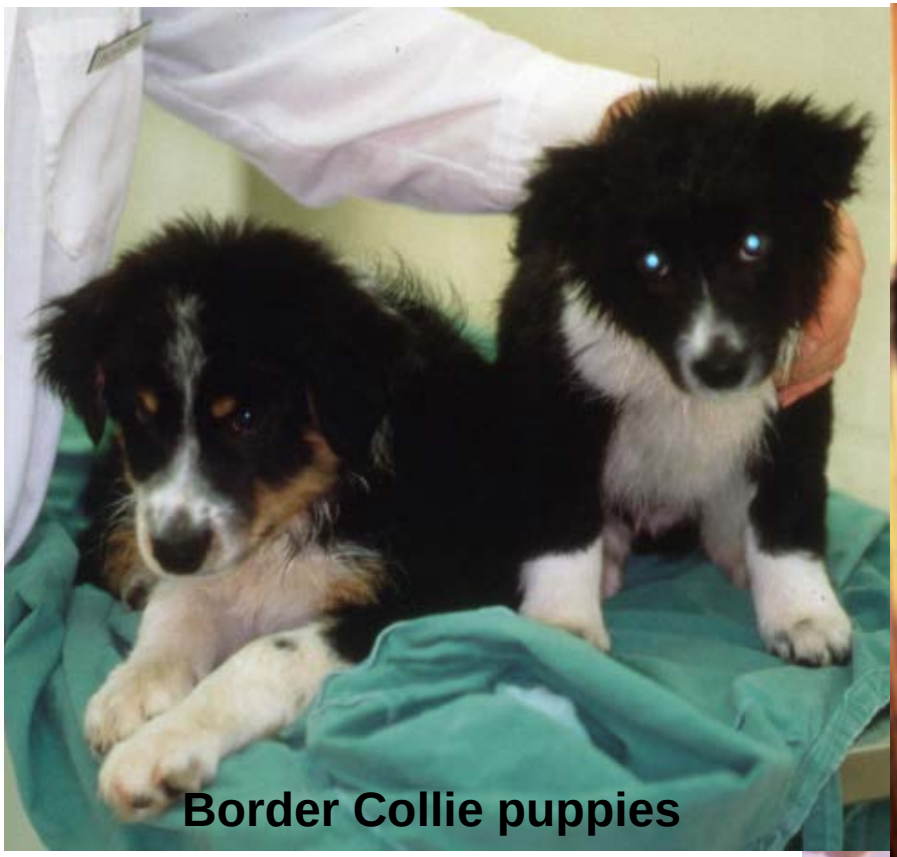
Glucose
Lactose
Fructose

Cystine
Ornithine
Taurine
Tyrosine
Sarcosine
Glycine

Citrulline
Glutamine
Alanine
Leucine
Valine
Lysine

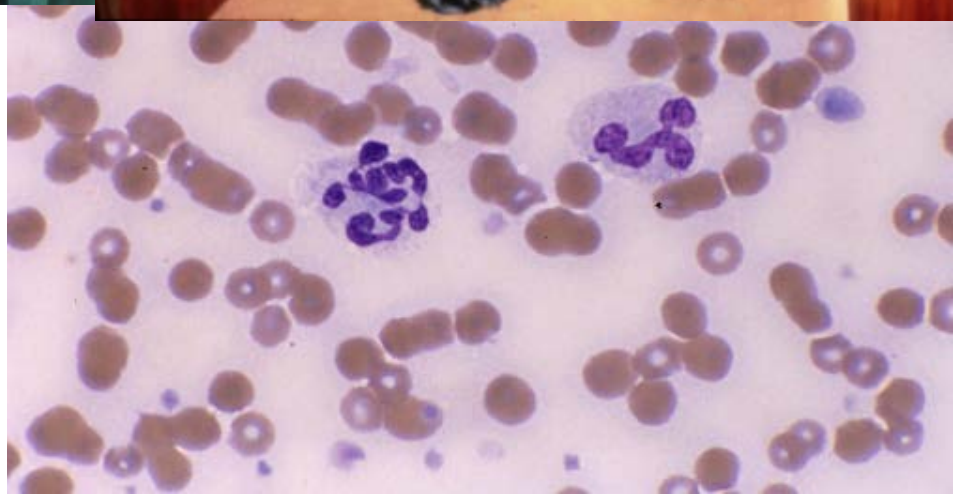
Arginine

Lactate
MMA
Isovaleric acid
Oxalate
Ketones



Border Collie puppies

"Vivian" Giant Schnauzer puppy



-Giant Schnauzers

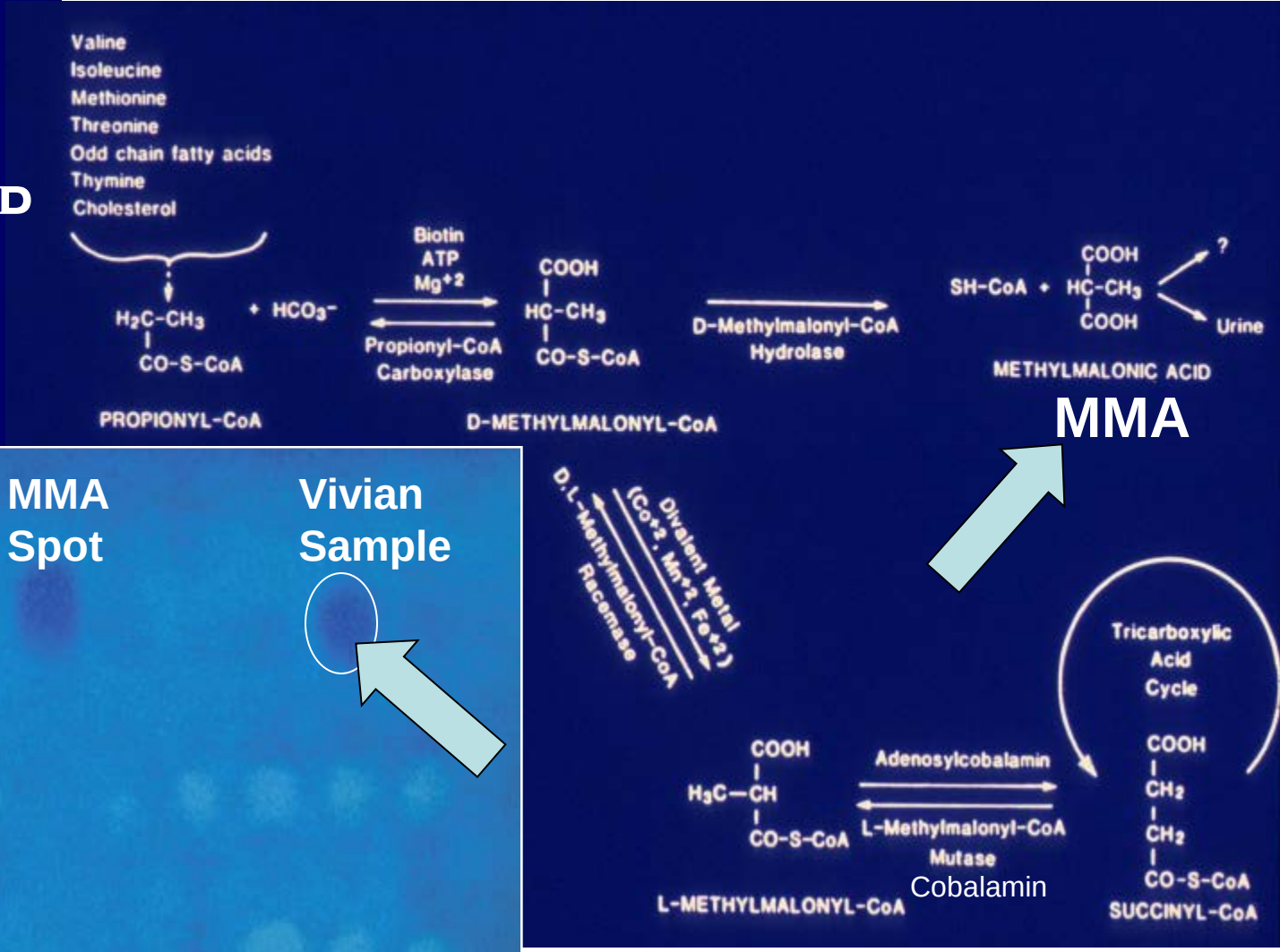
-Beagles

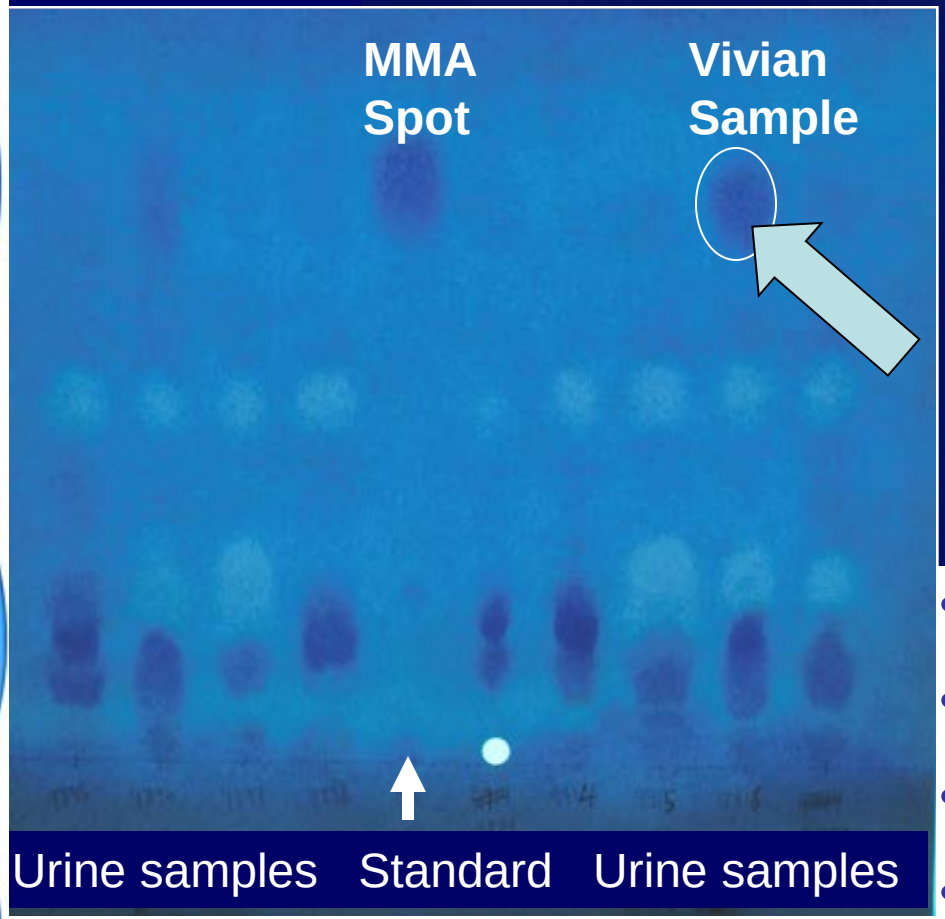
-Border Collies

-Australian Shepherds

-Kommodor -recently a cat

Urinary Organic Acid Screen





- Failing to thrive
- +/- Hyperammonemia
- Methylmalonic aciduria (MMA)
- Mild proteinuria

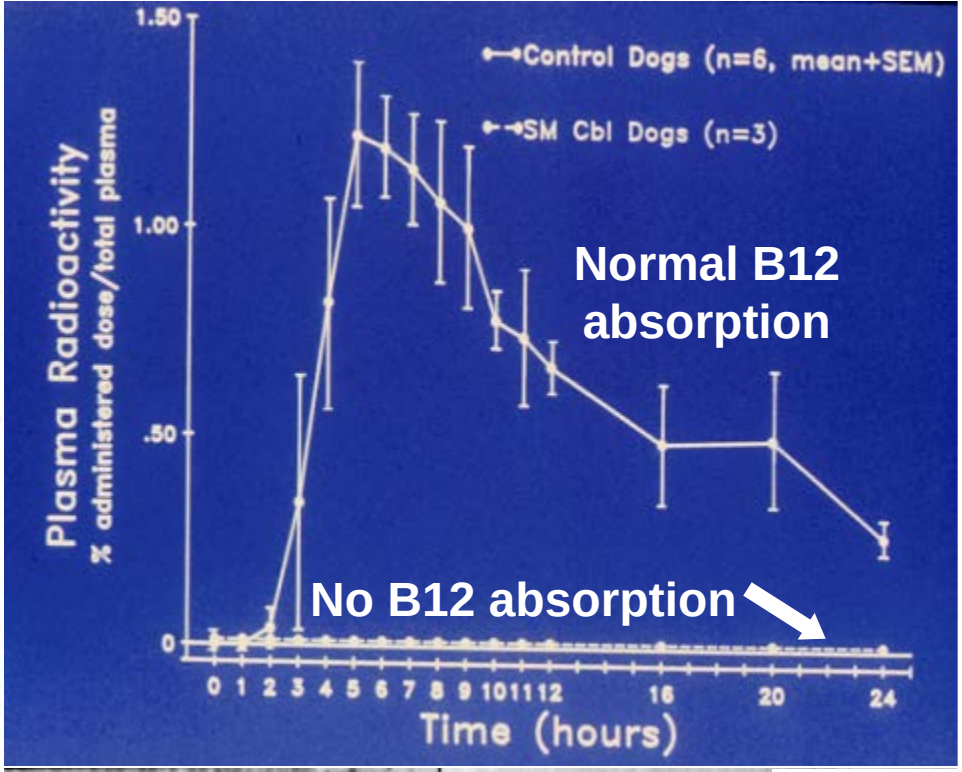
Cobalamin Malabsorption:

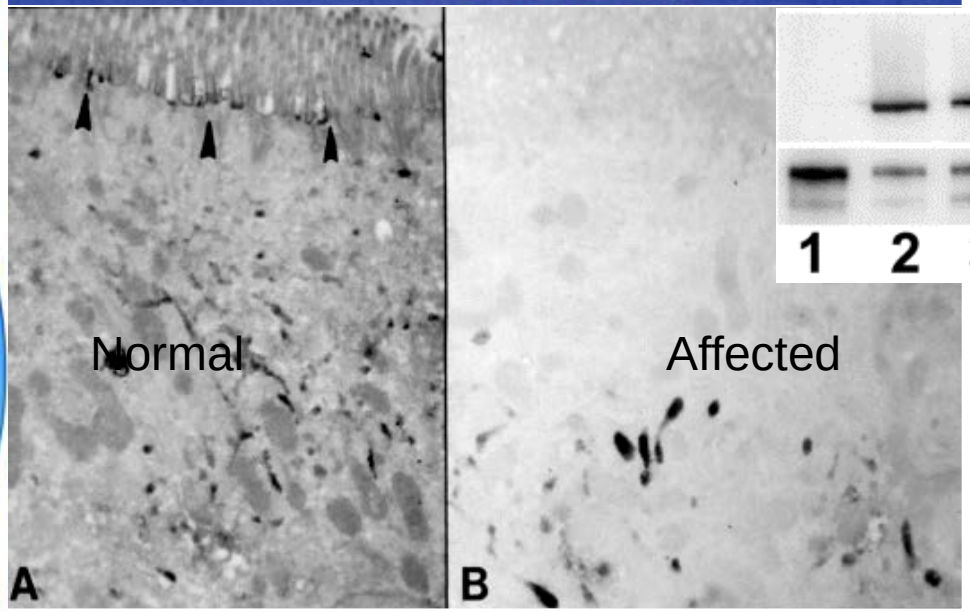
Serum cobalamin deficiency

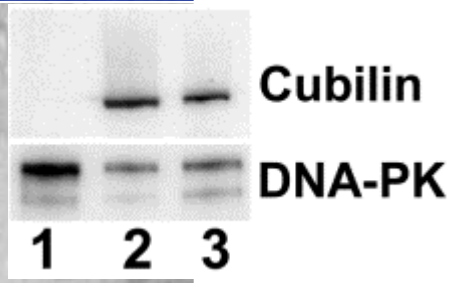
Ileum Receptor Defect

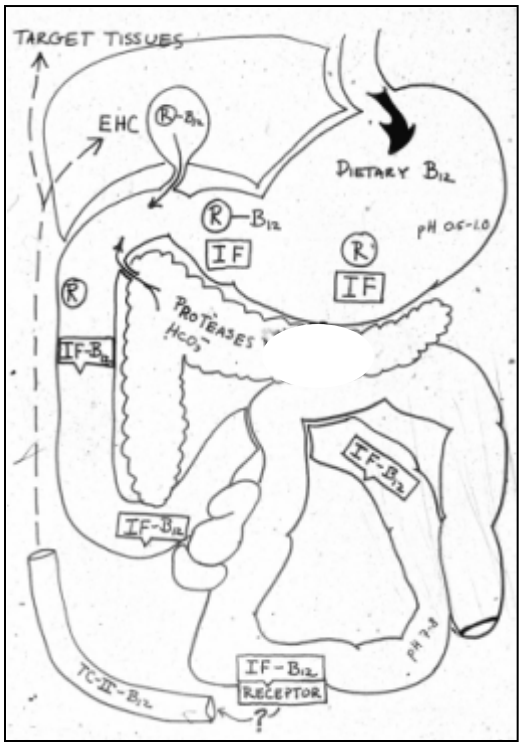
Cubulin and Amnionless protein

Autosomal-recessive trait





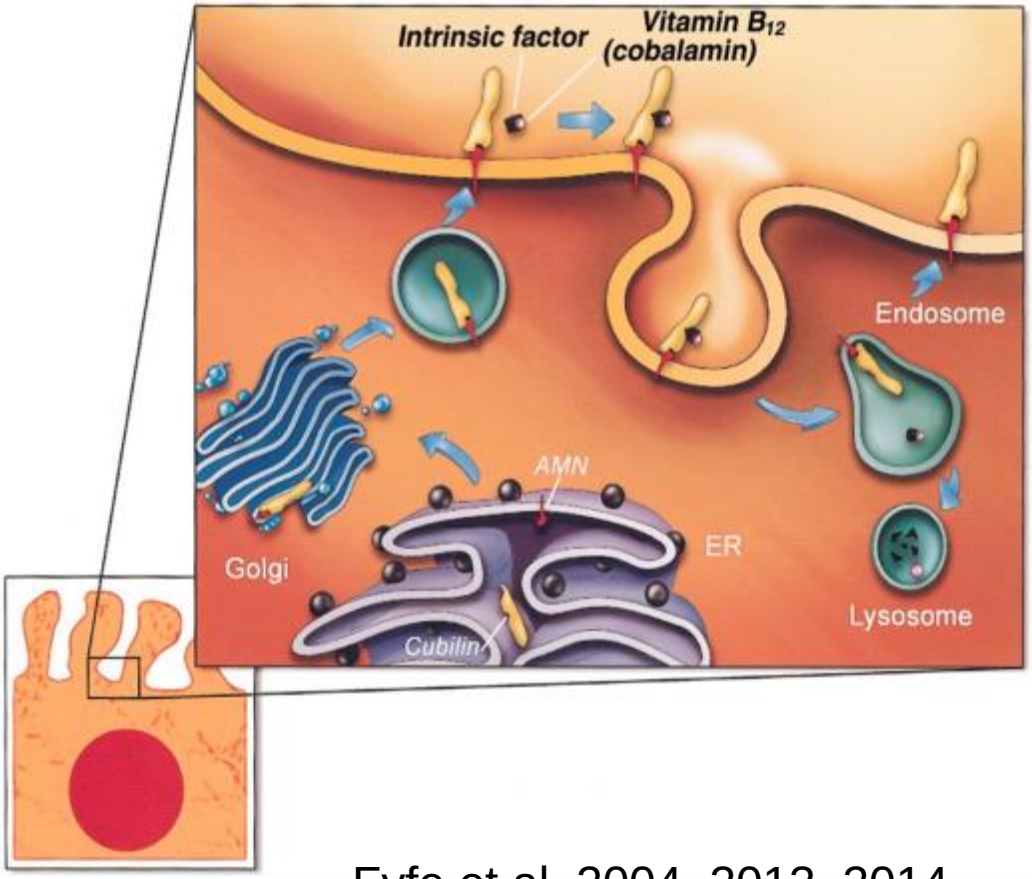




Vitamin B12 = Cobalamin

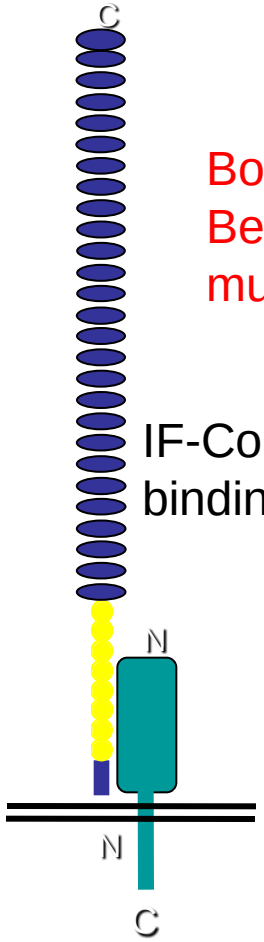
Selective Cobalamin Malabsorption

Amnionless (AMN) is required for Cubilin (CUBN) expression and endocytic function of Cubam



Fyfe et al, 2004, 2013, 2014

Border collies and Beagles have *CUBN* mutations

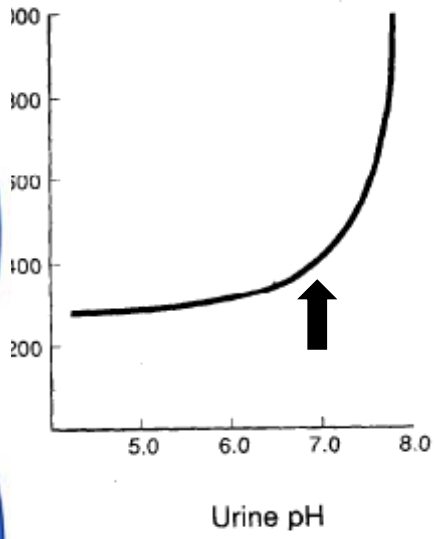


IF-Cobalamin binding site

Giant Schnauzers & Australian Shepherds have *AMN* mutations

- *Responsive to parenteral cobalamin administrations*
- *25 ug/kg subcutaneously*
  Every 2-3wks sc
  Simple life-long therapy
- *Good prognosis*

Cystinuria

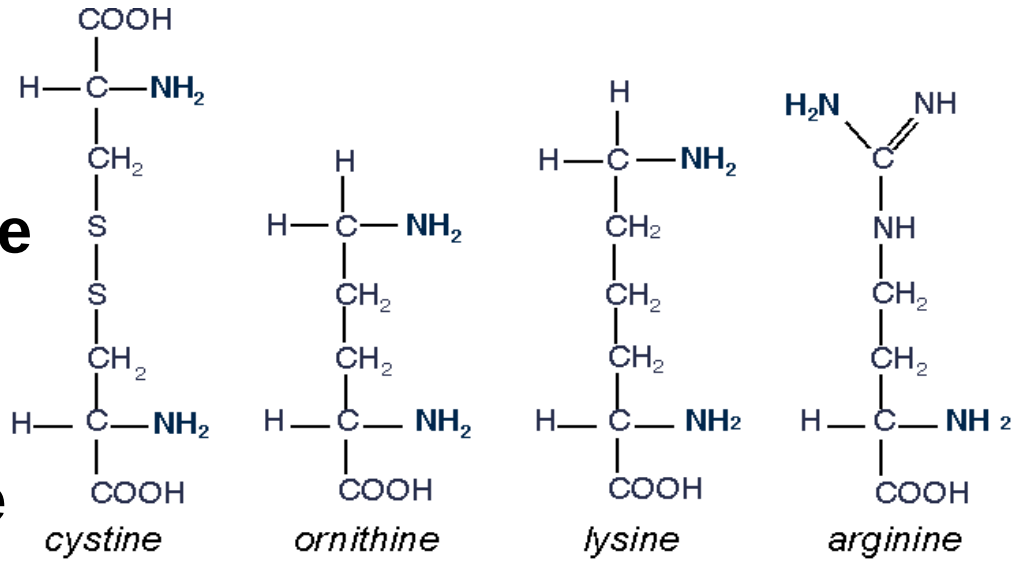


Cystine

Ornithine

Lysine

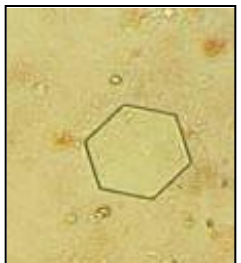
Arginine

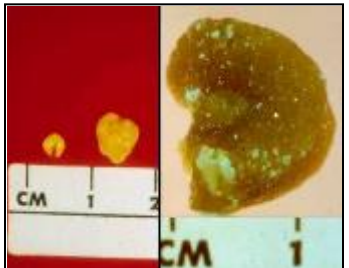


- Renal tubular and intestinal transport defect of cystine and dibasic amino acids (**COLA**)
- Cystine precipitates in slightly acidic urine.

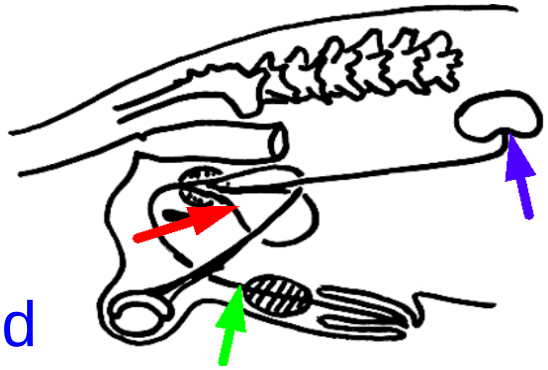
➡⬆ Cystine can lead to crystalluria & calculi formation & obstruction.

normal >99% resorption





Canine Cystinuria



1823 Lassaigne: First cystine calculi found

1935 Morris et al: Metabolic defect identified

1936 Green et al: Genetic basis in Irish Terriers (X-chrom.?)

1995 Autosomal-recessive trait in Newfoundlands

2000 Type I cystinuria caused by mutation SLC3A1 in Newfis

2013 Various mutations and androgen-dependent cystinuria

rBAT protein

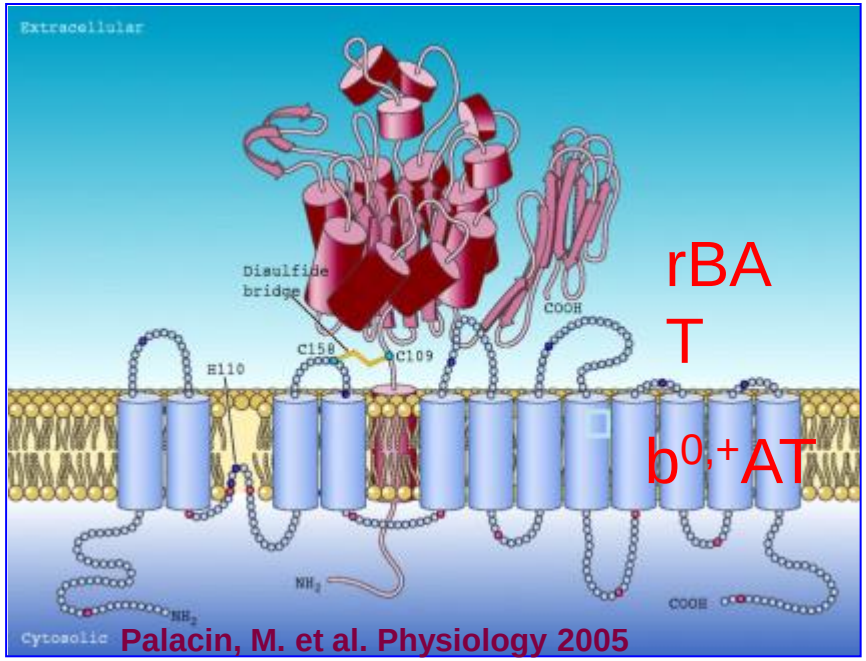
SLC3A1

Heavy chain

$b^{0,+}$AT protein

SLC7A9

Light chain



Palacin, M. et al. Physiology 2005

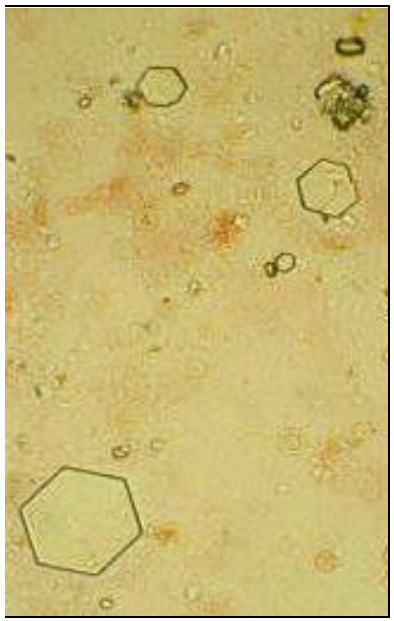
>70 Breeds with Cystinuria

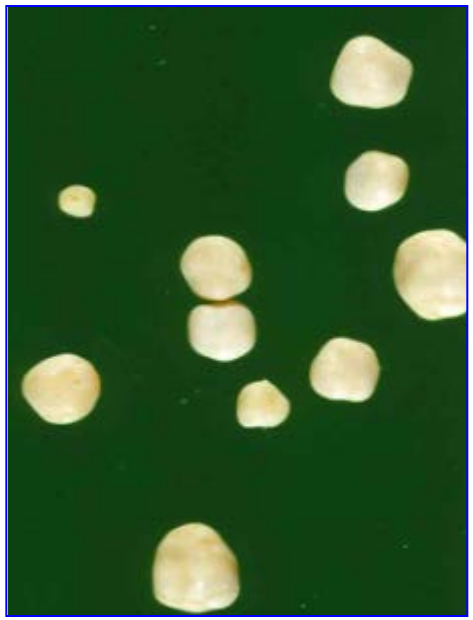
- Newfoundland
- Irish Terrier
- Mastiff
- English Bulldog
- Labrador Retriever
- Australian Cattle Dog
- Miniature Pinscher
- Basset Hound
- Dachshund

… and many others

Also common in humans: 1:7000

Diagnosis Cystinuria





3+ 4+

NEGATIVE POSITIVE

- Hexagonal crystals in acidic urine (highly variable)
- Yellow-brown calculi: Crystallography, chemical analysis
- Nitroprusside (cystine) test positive:
  - Always positive in type I & II cystinuria

	Cystine	***Ornithine***	***Lysine***	***Arginine***	***COLA***
Normal ***(µmol/g creatinine)***	≤178	≤100	≤200	≤100	≤500

Newfoundland & Landseer Type IA

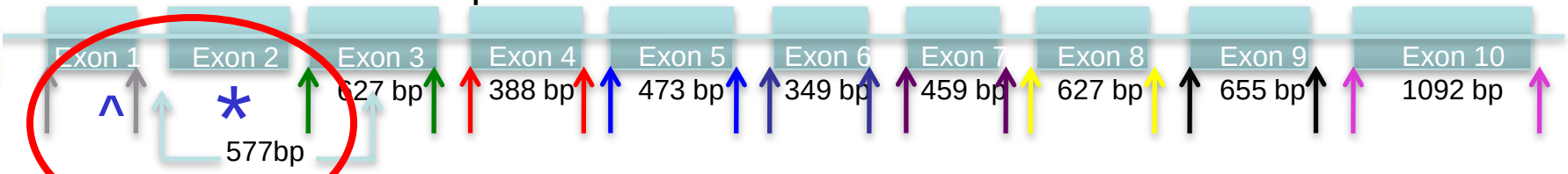
SLC3A1 (rBAT) Gene Mutation

- Coding sequence from cystinuric dogs compared to published canine genome sequence
- SLC3A1 (rBAT)

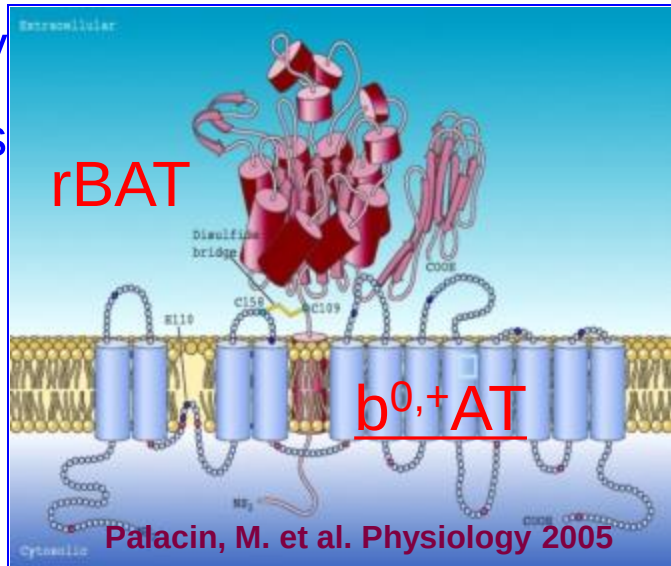
* Newfoundland and Landseer

^ Labrador Retriever mutation

Missense mutation: Stop codon



- Autosomal recessive inheritance definitively
- Stone formation in males usually by 2 years
- Males and females affected
- Affected popular sire in breed



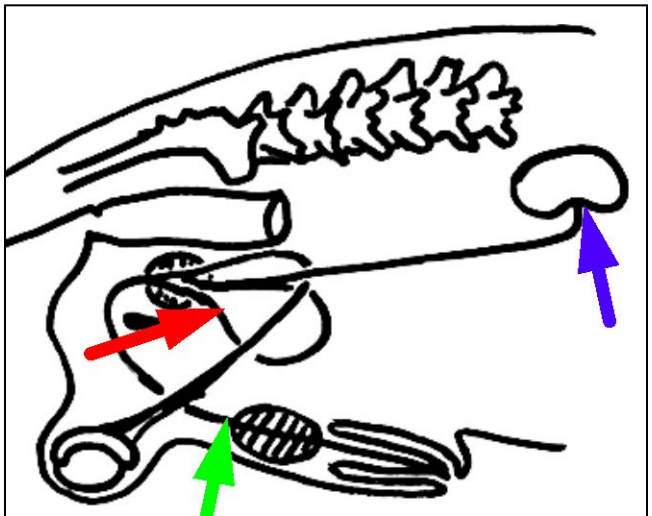
Cystinuria in Newfoundlands/Landseers

(Samples NOT representative of population)

Year	Affecteds %	Carriers %	Normal %	Mutant Allele
1996-99	2	26	72	15%
2000-03	0.5	18	82	9%
2004-08	0.3	4	94	2%
2009-13	0.1	0.3	99	0.2%

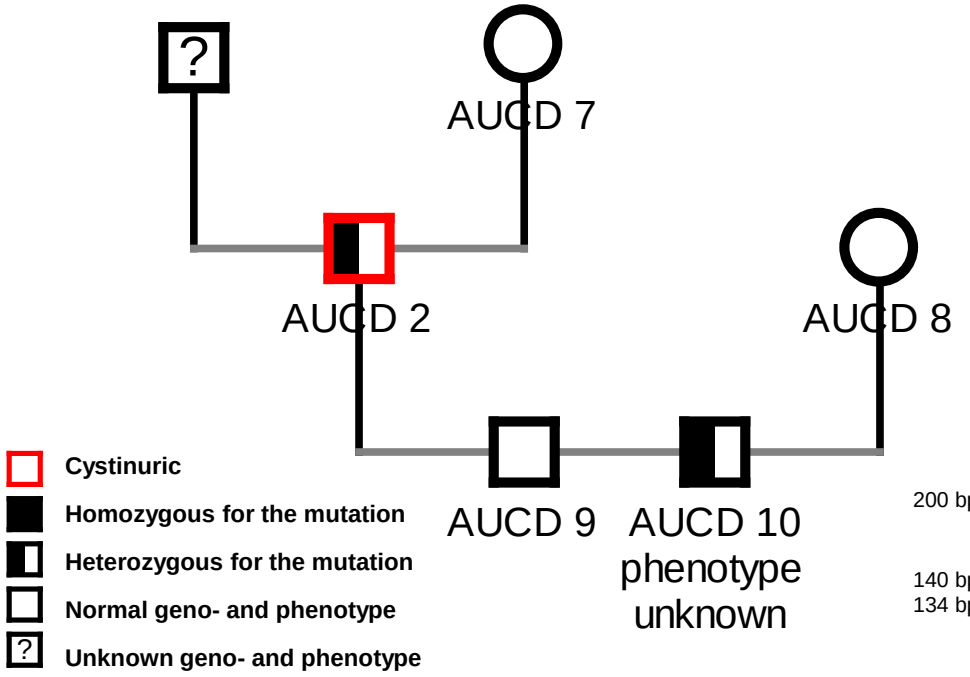
Canadian Champion Terry

PennGen screening test results; biased

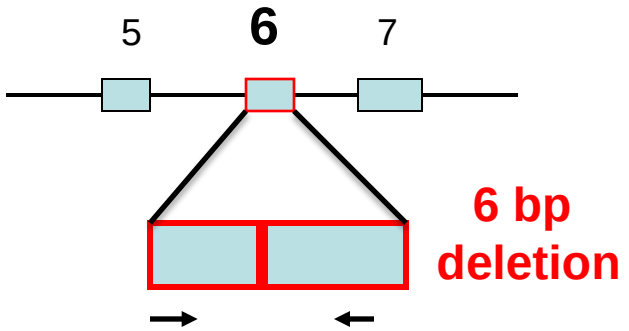


Australian Cattle Dog
"Blue Heeler" Type IIA

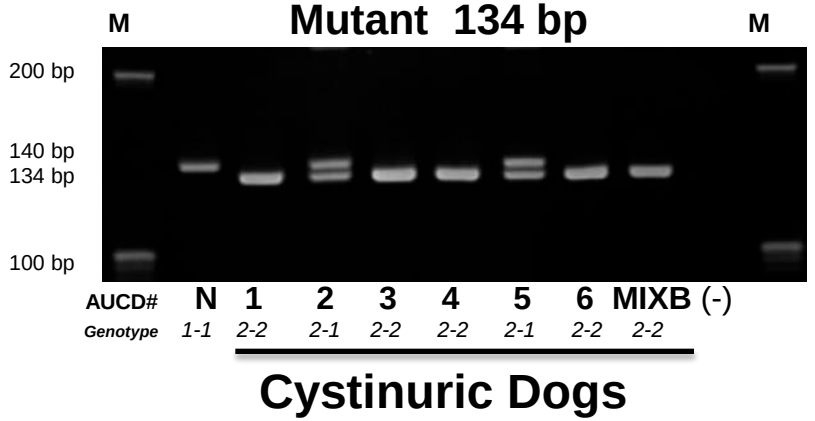
- **Homozygous** affected with a **severe phenotype**
- **Heterozygous** dogs with a **moderate cystinuria**



Mutation test by fragment length analysis:



Normal 140 bp
Mutant 134 bp



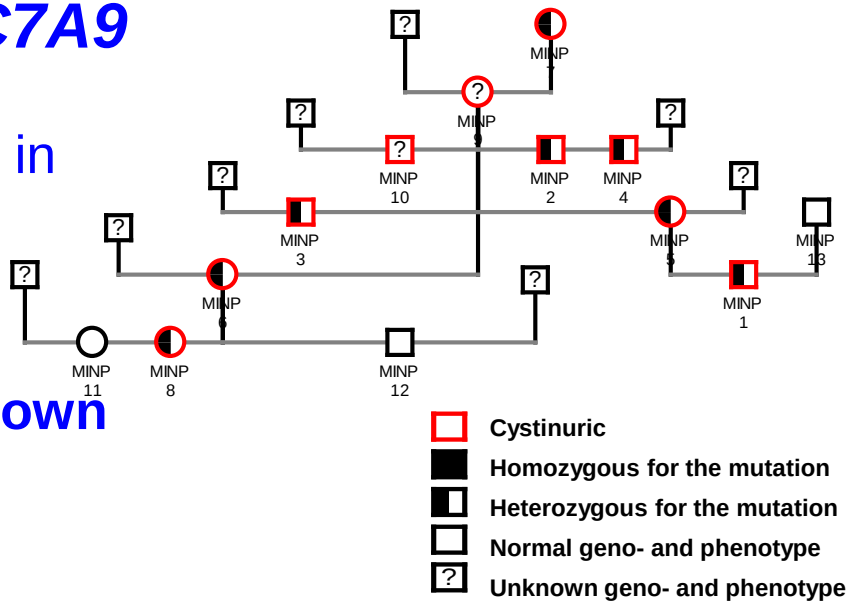
Autosomal dominant

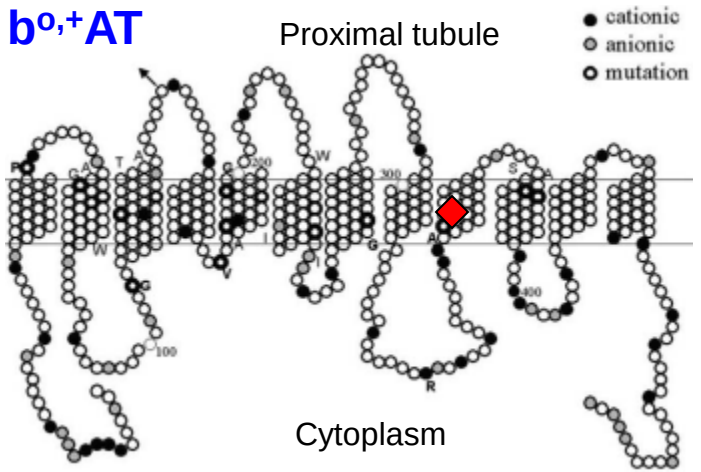
Miniature Pinschers – Type IIB

1st Missense Mutation in *SLC7A9*

- **Autosomal Dominant** (also typical in humans)
- Only **cystinuric heterozygotes** detected
- **Phenotype** of **homozygotes unknown**
- Missense **mutation** in one transmembrane domain (TMD) of $b^{o,+}AT$

  affects the amino acid **transport**
- **Similar mutations** in same TMD **in human patients**
- **Screening of dogs related to cystinuric family strongly recommended**





New Classification of Canine Cystinuria

Phenotype - Genotype		Type I A	Type IIA	Type IIB	Type III
Inheritance		**Autosomal recessive**	**Autosomal dominant**	**Autosomal dominant**	**Sex limited**
Gene		**SLC3A1**	**SLC3A1**	**SLC7A9**	**Undetermined**
Gender		*Males and Females*	*Males and Females*	*Males and Females*	*Intact Adult Males*
Androgen dependence		*No*	*No*	*No*	*Yes*
COLA *µmol/g creat.* ***(normal ≤ 500)***	**homozygous**	*≥ 8000*	*≥ 8000*	*unknown*	*≤ 4000*
	heterozygous	*≤ 500*	*≥ 3000*	*≥ 700*	
Breeds		*Newfoundland* *Landseer* *Labrador*	*Australian Cattle Dog*	*Miniature Pinscher*	*Mastiff & Related Breeds* *Scottish Deerhound* *Irish Terrier*

Cystinuria Type III

Cysinuric Maned Wolves

Also cats, ferrets and servals

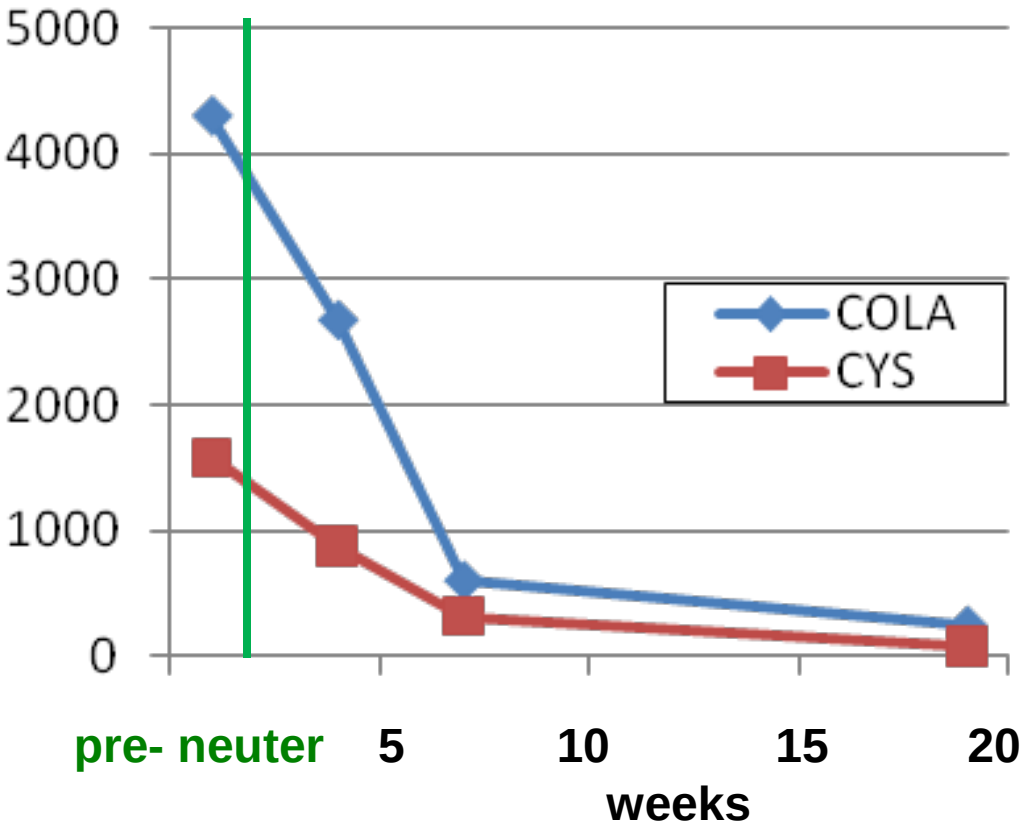
>70 canine breeds, frequently in Mastiffs, English Bulldogs, Bassets, Dachshunds, Irish Wolfhounds

Mature male animals (sex-limited)

Marker test for Mastiff/Bulldogs now available

Effect of Castration for Androgen-dependent Cystinuria

10 cystinuric male Irish Terriers



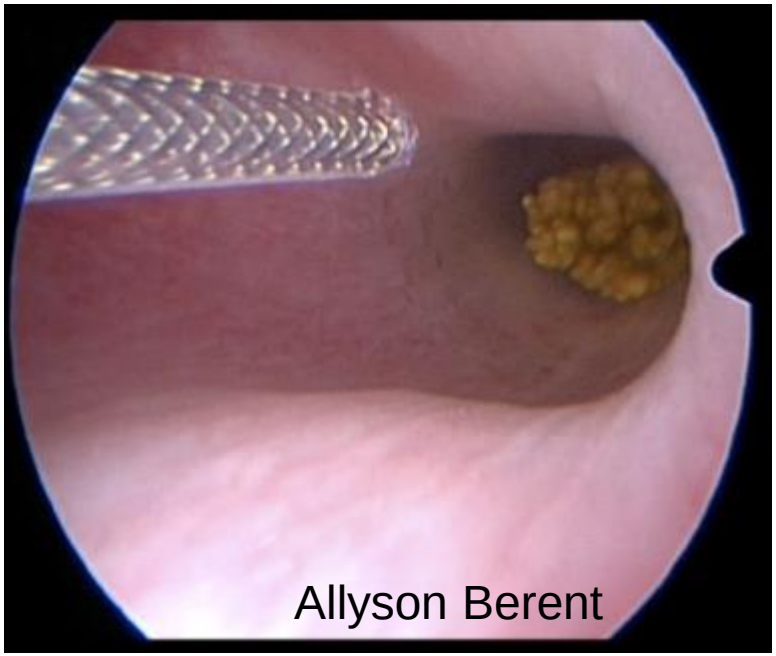
Following castration:
No further crystalluria & calculi formation

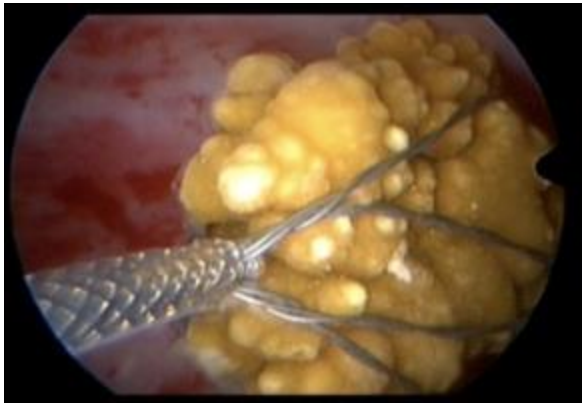
No known negative effect of androgene on renal amino acid transport

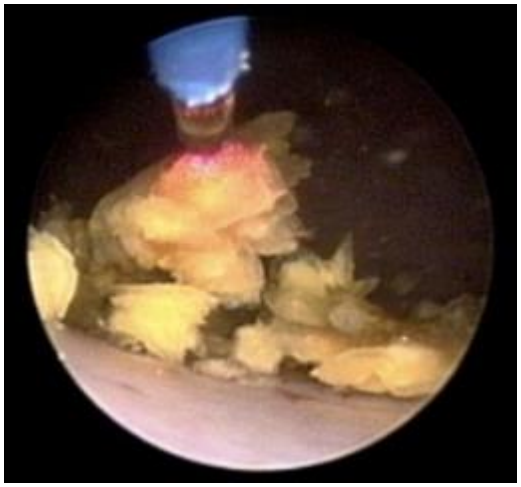
- **For type I & II cystinuria: no major change in COLA-uria**

Therapy for Cystinuria

- **Oft asymptomatic**
  - Preventative measures
- **Obstruction** (emergency)
  - Surgery
  - Endoscopy
  - Lithotripsy (soft calculi)
  - Preventative measures
- **Medical**
- **Castration**







Medical Management of Cystinuria

- **Urine alkalinization**
  - Bicarbonate
  - Potassium citrate
  - pH >7.5
- **Diet**
  - Special low protein diets
  - +/- alkalinization
  - No protein supplements
  - No amino acid suppl.
  - 

  - 
- **Diuresis**
  - Plenty of water
  - Frequent urination
  - No dehydration
- **Chelating Substances**
  - 2-MPG (Thiola)
  - D-Penicillamin
- **Infection prevention/ treatment**
  - Antibiotics postoperative
  - Optimal surgerical tech.

WSAVA Hereditary Disease Committee

World Small Animal Veterinary Association

Assisting clinicians with diagnosis, treatment and control of hereditary diseases and genetic predispositions in dogs and cats.

WSAVA
Global Veterinary Community

Associate POWERED by VIN

WALTHAM
Bringing the science to life.™

DNA Testing Laboratories

- 46 labs identified

  42 still offer tests

- 19 research; 23 commercial labs
  27 dogs only; 5 cats only; 10 both

- 151 mutations: 137 in dogs; 27 in cats

- 135 tests offered
  94 offered by multiple labs

- http://research.vet.upenn.edu/DNAGeneticsTestingLaboratorySearch/tabid/7620/Default.aspx

Slutsky et al. Veterinary J 2013.

Management of Hereditary Disorders

- Prevention of the production of affected animals is most important
- Control of further spread of mutant alleles
  - **maintain desirable traits and genetic diversity**

- Therapy is limited; there are ethical concerns
- Surgical interventions
- Supplementations
  - Vitamin B, Coagulation factors
- Symptomatic therapy
- Gene transfer experiments
  - Transplantations
  - Gene therapy

Control of Genetic Diseases

Considerations

- Severity of disease
- Onset of clinical signs
- Specific diagnosis
- Detection of carriers
- Accuracy of test
- Frequency of disease
- Breed gene pool
- Breed health club
- Registry
- Laws

Recommendations

- Do not breed affecteds
- Screen all breeders
- Breed clear to clear
- Breed clear to carriers
  - **Test all offspring**
  - **Select clear in next generation**
- Do not select only against one disease

Thank You!

Section of Medical Genetics

- *Faculty, Fellows & Residents*
- *Many Collaborators*
- *Referring Clinicians & Dog Owners*
- *Supported in part by National Institutes of Health (RR02512) and Canine Health and other Foundations*

penngen@vet.upenn.edu

http://www.vet.upenn.edu/penngen

PennGen