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
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

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.
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

- 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

CATCTTTTATCG

Dog 1

Dog 2

Dog 3

A – Adenine
G – Guanine
C – Cytosine
T – Thymine
SNPs = Single Nucleotide Polymorphisms (170K)

On 38 canine chromosome pairs

Green bars: Screening thousands of SNPs to look for variation
Genomic Approach to Finding a Trait

Clinical Status | Genotype - Deletion |
--- | --- | --- | --- | ---
 | del/del | del/wt | wt/wt | Total |
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

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

Amylase and maltase

Population Structure of Dog Breeds

Ancient/Asian

Herding

Modern/Hunting

Mastiff

Ostrander, 2007 & 2012
IGF1 allele is a major determinant of small size by genomics and candidate gene approach.

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 the 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

1990 – 3 mutations
2013 – 174 mutations

~230 Hereditary Diseases in Cats
2013 – 24 mutations

“Inherited Diseases in Dogs” [IDID], [http://www.vet.cam.ac.uk/idid](http://www.vet.cam.ac.uk/idid)

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.

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 \( \rightarrow \)

Functional laxity

Excess stress on cartilagenous structures

Microfractures, release of inflammatory mediators

Periarticular osteophytosis, sub-chondral bone sclerosis, joint remodeling

Osteoarthritis
**Hip Dysplasia & Laxity**

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

![Norberg Angle ≥ 105°](image)

![Compression view](image)

![Distraction view](image)

![Smith & PennHIP](image)
Osteoarthritis & Diet

Radiographic OA

- Blue line: Control
- Red line: Restricted

OA-free Interval

Years

Control Fed
BCS 6.7

Restricted Fed
BCS 4.6

Smith et al. JAVMA
False-negative diagnosis
( Normal phenotype but bad genes )

Gene pool

True-negative diagnosis
( Normal phenotype but good genes )

Diagnostic test

True-positive diagnosis
( Diseased phenotype but bad genes )

Do not breed
( Discarded genes )

False-positive diagnosis
( Diseased phenotype but good 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

Small Animal Hospital
~23,000 animals/year
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.

Metabolic consequences in a pathway

- Enzyme deficiencies
- Structural proteins
- Receptors, adhesion molecules, ion channels
- Plasma proteins
Metabolic Genetic Screening Tests

LIQUID SAMPLE

URINE

SERUM

WBC granulations

Severe lipemia
Metabolic Genetic Screening Tests

Urine

Spot Tests
- MPS spot
- Clinitest
- Ketostix
- Nitroprusside test

Paper Chromatography
- Amino Acid
- Organic Acid
- Amino acid analyzer
- MS/Gas chromatography

MPS electrophoresis

Carbohydrate chromatography
- Chondroitin sulfate
- Dermatan sulfate
- Heparin sulfate
- Keratan sulfate
- Glucose
- Lactose
- Fructose
- Cystine
- Ornithine
- Taurine
- Tyrosine
- Sarcosine
- Glycine
- Arginine
- Citrulline
- Glutamine
- Alanine
- Leucine
- Valine
- Lysine
- Lactate
- MMA
- Isovaleric acid
- Oxalate
- Ketones

Metabolic Genetic Screening Tests

Chondroitin sulfate
Dermatan sulfate
Heparin sulfate
Keratan sulfate
Glycine
Lysine
Arginine

MPS electrophoresis
Giant Schnauzers
Beagles
Border Collies
Australian Shepherds
Kommodor
recently a cat

“Vivian” Giant Schnauzer puppy

Border Collie puppies
- Failing to thrive
- +/- Hyperammonemia
- Methylmalonic aciduria (MMA)
- Mild proteinuria
Cobalamin Malabsorption:

Serum cobalamin deficiency

Ileum Receptor Defect
Cubulin and Amnionless protein

Autosomal-recessive trait
Amnionless (AMN) is required for Cubilin (CUBN) expression and endocytic function of Cubam.

Border collies and Beagles have *CUBN* mutations.

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
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.
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

b₀⁺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

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

<table>
<thead>
<tr>
<th>Cystine (µmol/g creatinine)</th>
<th>Ornithine</th>
<th>Lysine</th>
<th>Arginine</th>
<th>COLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>≤178</td>
<td>≤100</td>
<td>≤200</td>
<td>≤100</td>
</tr>
</tbody>
</table>
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 demonstrated
  - Stone formation in males usually by 2 years
  - Males and females affected
  - Affected popular sire in breed

Palacin, M. et al. Physiology 2005

b0,+AT

rBAT

Exon 1: 577bp
Exon 2: 627 bp
Exon 3: 388 bp
Exon 4: 473 bp
Exon 5: 349 bp
Exon 6: 459 bp
Exon 7: 627 bp
Exon 8: 655 bp
Exon 9: 1092 bp
Exon 10: 1092 bp
Cystinuria in Newfoundland/Landseers
(Samples NOT representative of population)

<table>
<thead>
<tr>
<th>Year</th>
<th>Affecteds %</th>
<th>Carriers %</th>
<th>Normal %</th>
<th>Mutant Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996-99</td>
<td>2</td>
<td>26</td>
<td>72</td>
<td>15%</td>
</tr>
<tr>
<td>2000-03</td>
<td>0.5</td>
<td>18</td>
<td>82</td>
<td>9%</td>
</tr>
<tr>
<td>2004-08</td>
<td>0.3</td>
<td>4</td>
<td>94</td>
<td>2%</td>
</tr>
<tr>
<td>2009-13</td>
<td>0.1</td>
<td>0.3</td>
<td>99</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>AUCD#</th>
<th>N</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>MIXB (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>1-1</td>
<td>2-2</td>
<td>2-1</td>
<td>2-2</td>
<td>2-2</td>
<td>2-1</td>
<td>2-2</td>
<td>2-2</td>
</tr>
</tbody>
</table>

- Normal 140 bp
- Mutant 134 bp
- 6 bp deletion

Autosomal dominant

Cystinuric Dogs
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

<table>
<thead>
<tr>
<th>Phenotype - Genotype</th>
<th>Type I A</th>
<th>Type IIA</th>
<th>Type IIB</th>
<th>Type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inheritance</td>
<td>Autosomal recessive</td>
<td>Autosomal dominant</td>
<td>Autosomal dominant</td>
<td>Sex limited</td>
</tr>
<tr>
<td>Gene</td>
<td>SLC3A1</td>
<td>SLC3A1</td>
<td>SLC7A9</td>
<td>Undetermined</td>
</tr>
<tr>
<td>Gender</td>
<td>Males and Females</td>
<td>Males and Females</td>
<td>Males and Females</td>
<td>Intact Adult Males</td>
</tr>
<tr>
<td>Androgen dependence</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>COLA (μmol/g creat. (normal ≤ 500))</td>
<td>homozygous</td>
<td>(\geq 8000)</td>
<td>(\geq 8000)</td>
<td>unknown</td>
</tr>
<tr>
<td></td>
<td>heterozygous</td>
<td>(\leq 500)</td>
<td>(\geq 3000)</td>
<td>(\geq 700)</td>
</tr>
<tr>
<td>Breeds</td>
<td>Newfoundland Landseer Labrador</td>
<td>Australian Cattle Dog</td>
<td>Miniature Pinscher</td>
<td>Mastiff &amp; Related Breeds Scottish Deerhound Irish Terrier</td>
</tr>
</tbody>
</table>
Cystinuria Type III

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

Mature male animals (sex-limited)

Marker test for Mastiff/Bulldogs now available

Cysinuric Maned Wolves
Also cats, ferrets and servals
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

Allyson Berent
Medical Management of Cystinuria

- Urine alkalization
  - Bicarbonate
  - Potassium citrate
  - pH >7.5

- Diet
  - Special low protein diets
  - +/- alkalization
  - 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.
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](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