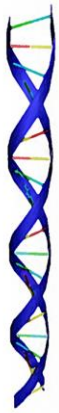
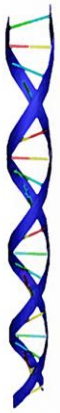


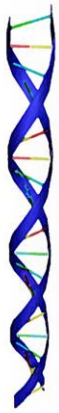
Genetic Testing: Understanding Results & Putting Them Into Practice

Kari J Ekenstedt, DVM, PhD
Katie Minor, RN
College of Veterinary Medicine
University of Minnesota
April 29th, 2016



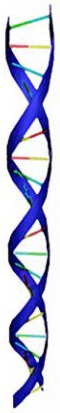
Outline

- 1. Primer
  - General Genetics "Stuff"
  - The UMN Canine Genetics Lab Website and Sample Submission Basics
- 2. LPN1, LPN2, LEMP (Breeding Decisions)
- 3. New Technologies
- 4. Q&A



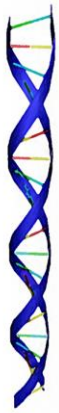
Review Terms

- Gene: Unit of inheritance; sequence of nucleotides (bases) that, when properly transcribed, spliced, and translated, becomes a protein that performs a function in the body
- Allele: A version of a gene
- Homozygote: Having identical alleles for a given gene
- Heterozygote: Having different alleles for a given gene



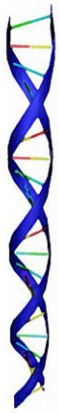
Review Terms

- Genotype: Actual alleles an individual has for a gene
- Phenotype: Expressed/observed trait or disease state
  - A phenotype is not always equivalent to a genotype
- Autosome: Any chromosome not involved with sex determination
  - Genes on autosomes are equivalent in males and females
  - The only chromosomes that are NOT autosomes are the sex chromosomes



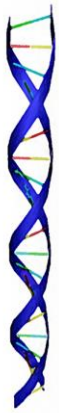
Review Terms

- Dominant (mutation): Requires only one allele of a given gene to determine the phenotype
- Recessive (mutation): Must be present in the homozygous state to be expressed in the phenotype
  - Carriers (or heterozygotes) of recessive alleles are phenotypically normal



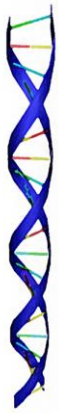
Review Terms

- Penetrance: Degree to which the affected genotype correlates to the phenotype
  - Or, the proportion of individuals who have a particular genotype that also express the phenotype
- Haplotype: set of DNA variations that tend to be inherited together
  - A set of alleles on the same chromosome



Types of DNA-Based Tests

- Mutation Test
  - Exact alteration in nucleotide sequence is tested for
  - May be in many breeds or isolated to one breed
- Marker Test
  - DNA-differences *near* the disease-causing alteration, but do not test for the mutation itself
    - Sure of area/locus, but the exact mutation hasn't yet been identified
    - A genetic marker "tags" the region (most of the time)
  - Not transferrable between breeds

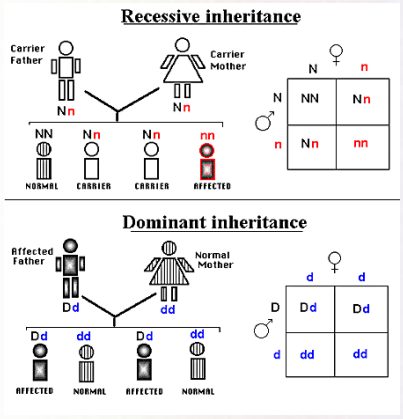


Simple Modes of Inheritance

- Simple: Mendelian modes of inheritance
  - Recessive
    - Autosomal recessive inherited diseases are the most common type of single-gene disorders in dogs and cats
      - Usually from inbreeding
    - Animal must inherit two copies of the mutant allele, one from each parent, to have the altered phenotype
      - Animal with one copy of the mutant allele (a heterozygote or carrier) or an animal with two copies of the normal (non-mutant) allele has normal phenotype

Simple Modes of Inheritance

- Simple: Mendelian modes of inheritance, cont.
  - Dominant
    - Only one copy of the mutant allele, which can come from the sire or the dam, is needed to make the altered phenotype
  - Sex-linked
    - Specifically on the sex chromosome(s)
- Even simple isn't simple
  - Partial/incomplete penetrance, etc.



Note: The letters here (D's, N's) have nothing to do with the LPN1 and LPN2 tests in Leos – they are just random letters used in this human example

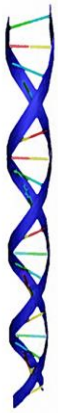
http://www.accessexcellence.org/RC/VL/GG/recessive.php

Recessive Trait

- Not easy to identify
  - Parents are not affected
- Several thousand recessive traits have been observed in humans
  - Dogs are getting closer to that number
- Rare, recessive traits are more likely to appear when parents are related to one another
- In animal breeding, as we try to improve a breed, we mate animals that are more closely related to one another

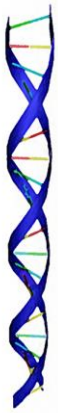
Autosomal Recessive Inheritance

- As long as the frequency of the mutant allele for a disorder is low, it may lurk in a breed for many generations…
  …only to appear when, by chance, two carriers are mated, and affected individuals are observed in a litter
- Frequency of the affected allele can become very high
  - Popular sire effect
- Hard to eradicate without a genetic test
  - Carriers are clinically normal



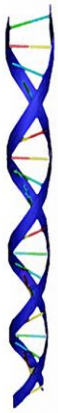
Dominant Trait

- Easier to identify – every individual with the allele will manifest the trait
  - Easy to trace through the generations
- Every affected individual should have at least one affected parent
- Extremely rare: brand new dominant mutation
  - ~ one in a million



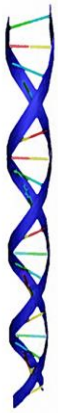
Sex-Linked Genes

- Sex-Linked Genes are on the X or Y chromosome
  - Called "X-Linked" when on the X
- Example: Hemophilia – an X-linked blood clotting disorder (often called Hemophilia A or "royal hemophilia")
  - (NOTE: there are other blood clotting disorders that are not x-linked)



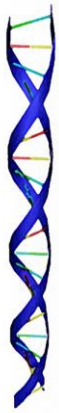
Hemophilia – an X-Linked Disease

- Unable to clot blood appropriately
  - Cuts, bruises, wounds = bad news!
  - Keep bleeding…can die!
    - Used to be lethal - they'd die very young
    - Today, therapies to help them clot
- Most of the affected individuals are male



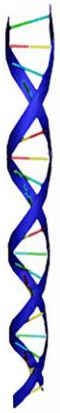
Sex-Linked Genes - Hemophilia

- Russian imperial family around the 1900s
  - Four daughters, one son
    - Son (Alexis) affected with hemophilia
    - Alexis affected because his mom was a heterozygous carrier
    - SHE got it from her mother, who got it from Queen Victoria of England
      - May have been new mutation in Queen Victoria, or from earlier than that



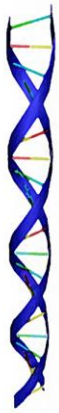
Penetrance

- So far, we have assumed genotype can always predict phenotype
  - FULLY PENETRANT
- When the genotype does NOT produce the expected phenotype = INCOMPLETE PENETRANCE



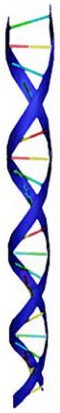
Incomplete Penetrance

- Human polydactyly (extra fingers and toes)
- Usually caused by a dominant allele
- For example: Individual A has a parent with polydactyly, and has kids with polydactyly, but Individual A has exactly 10 toes/fingers themselves
  - Incompletely penetrant in Individual A



Penetrance

- **INCOMPLETE PENETRANCE:** Despite having the appropriate genotype, an individual does not express the trait
- The road from genotype to phenotype is subject to modification



Complex Inheritance

- Polygenic diseases
  - Controlled by any number of genes
    - Each with a small, additive effect
    - There could also be genes contributing a "protective" effect (something that decreases risk)
  - Often have significant interaction with environmental factors
  - Canine examples: Hip Dysplasia, Cancers, Allergies, Diabetes Mellitus, Cryptorchidism, etc.
- Unknown
  - Often the mode of inheritance isn't established
  - "Breed predisposition"
- MUCH harder to make breeding decisions for these!

Dog Genome Facts

- Average mammalian genome is 3 billion base pairs (so, 6 billion nucleotides or “letters”)
- Average mammalian genome has 20,000 genes

Leonbergerclubofamerica.com

UMN Canine Genetics Laboratory Website

http://z.umn.edu/caninegenetics

Leonberger-Specific Website

- http://z.umn.edu/leonberger

UMN Canine Genetics Laboratory Website

- Access submission forms
  - Including instructions for submission
- Our mailing address has changed!!
- Things to pay attention to:
  - Sample type being submitted
    - How to get them right!
  - Shipping considerations
    - How to save money!
    - How to not wreck your sample!

Sample Types

- Cheek Swab
  - Cheek cells exfoliate onto swab
  - Best to isolate from other dogs and food for ~ 1 hour prior to swab
- Blood
  - White blood cells provide DNA
  - Need 1-3 mL of blood
- Tissue
- Dew claws (not just the nail, need skin and other tissue that is removed when the dew claw is removed)
- Semen

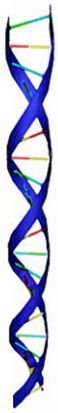
Cheek Swabs

- Commonly submitted for genetic testing
- VIDEO
  - https://www.youtube.com/watch?v=ZiBEM_0Qq3I
- Things to note:
  - Swabbing puppies that are still nursing is not recommended
    - If you must swab a nursing litter, isolate them for at least one hour from the dam and littermates
    - Dam's cells can get into their oral cavities
  - Consider sending two sets, collected 1 day apart (ship together)
  - Ship swabs in an ENVELOPE (not in sealed plastic – tube, bag – they will turn moldy!)

Dirty Cheek Swab

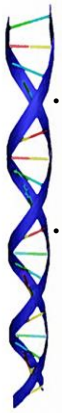
Food Particles

Moldy Cheek Swab



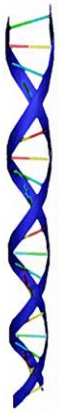
Blood

- Commonly submitted for research projects and DNA archiving
- Follow instructions for specific submissions
  - BUT, typically EDTA (non-clot) tubes
    - In USA, purple-top tube
  - 1-3 mL blood, up to 10mL
    - Puppies generally need to be 5-6 weeks old to be big enough to lose 1-3 mL blood without problems



Cheek Swab vs. Blood

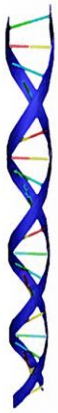
- Pros:
  - Can do at home
  - Cheap
  - Easy to ship
- Cons:
  - Can be contaminated
  - Don't always work for testing – result delays
  - Lower yields & lower quality for archiving
    - Avg. yield 300 - 1,000 ng
- Cons:
  - Trip to vet's office
  - Blood draw fees
  - Challenging to ship if outside the US
- Pros:
  - No risk of contamination
  - Almost always work
  - High yields & high quality for archiving
    - Avg. yield >75,000 ng



Shipping
How to Save Money!

- Cheek Swabs
  - Ship in paper/cardboard envelope
    - Plastic is the enemy of cheek swabs!
  - US Mail is fine
- Blood
  - Protect your sample-- a pill bottle works great
  - Cool pack only needed if it's hot ≥80 F
  - US Mail is fine
    - Should ship for < $10 in a small box
    - Vet office will charge a premium to do the shipping for you
  - Write "Exempt Animal Specimen" on outside

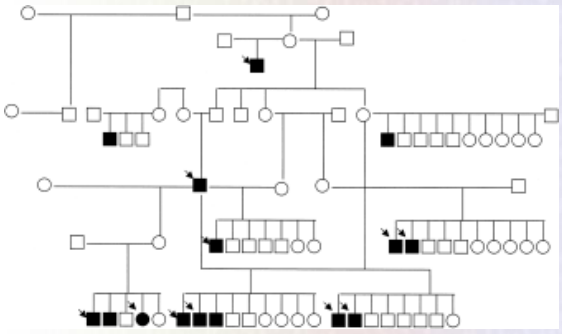
* Don't forget to save your tracking number



Health Updates to Our Lab

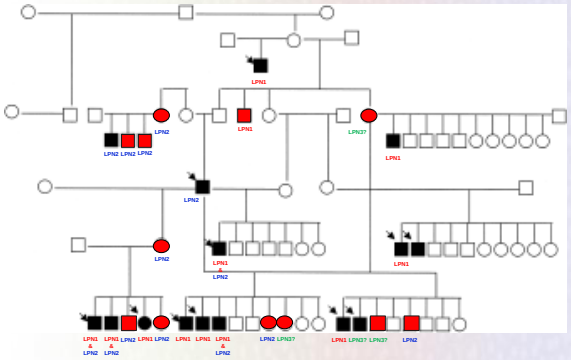
- To us, a dog is only as old as their last health update
  - A dog is not a control until we have health information through age 8 yrs.
- This information helps us provide you with the best breeding recommendations
- We want to know if any clinical signs have changed, diseases developed or progressed, etc.

Inherited polyneuropathy in Leonberger dogs: A mixed or intermediate form of Charcot-Marie-Tooth disease?

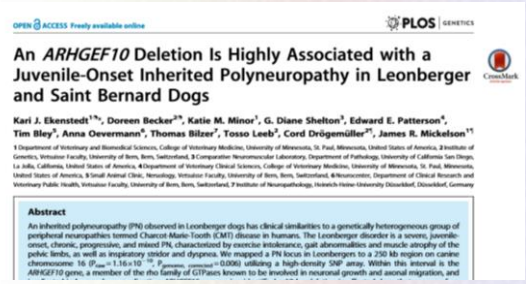


Muscle & Nerve
Volume 27, Issue 4, pages 471-477, 25 FEB 2003 DOI: 10.1002/mus.10350
http://onlinelibrary.wiley.com/doi/10.1002/mus.10350/full#fig3

Same Pedigree Today - Health Updates Matter!



Leonberger-Specific: LPN1



OPEN ACCESS Freely available online — PLOS | GENETICS

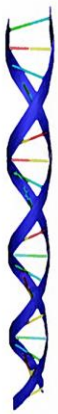
An *ARHGEF10* Deletion Is Highly Associated with a Juvenile-Onset Inherited Polyneuropathy in Leonberger and Saint Bernard Dogs

Kari J. Ekenstedt[1¤a], Doreen Becker[2¤b], Katie M. Minor[1], G. Diane Shelton[3], Edward E. Patterson[4], Tim Bley[5], Anna Oevermann[6], Thomas Bilzer[7], Tosso Leeb[2], Cord Drögemüller[2¤], James R. Mickelson[1¤]

1 Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, United States of America, 2 Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, 3 Comparative Neuromuscular Laboratory, Department of Pathology, University of California San Diego, La Jolla, California, United States of America, 4 Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, United States of America, 5 Small Animal Clinic, Neurology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, 6 Neurocenter, Department of Clinical Research and Veterinary Public Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland, 7 Institute of Neuropathology, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

Abstract

An inherited polyneuropathy (PN) observed in Leonberger dogs has clinical similarities to a genetically heterogeneous group of peripheral neuropathies termed Charcot-Marie-Tooth (CMT) disease in humans. The Leonberger disorder is a severe, juvenile-onset, chronic, progressive, and mixed PN, characterized by exercise intolerance, gait abnormalities and muscle atrophy of the pelvic limbs, as well as inspiratory stridor and dyspnea. We mapped a PN locus in Leonbergers to a 250 kb region on canine chromosome 16 ($P_{raw} = 1.16 \times 10^{-10}$, $P_{genome,\ corrected} = 0.006$) utilizing a high-density SNP array. Within this interval is the *ARHGEF10* gene, a member of the rho family of GTPases known to be involved in neuronal growth and axonal migration, and



Leonberger-Specific: LPN1

- Peripheral neuropathy
- Affected dogs can show signs before one year of age
- Weakness, muscle atrophy, gait abnormalities
  - Worse on hind limbs
- May also have laryngeal paralysis
- Some have shortened lifespan
  - Become non-weight-bearing
  - Severe respiratory difficulties

LPN1

- Mutation Test
  - Mutation is in gene called *ARHGEF10*
    - Mutations in this gene cause neurological abnormalities in people, too
  - 10 bp deletion
- Autosomal Recessive
  - Carriers are generally considered healthy
    - Few with signs, may have other polyneuropathy?
- Breeding decisions
  - Want to prevent affected puppies
    - No carrier-to-carrier breedings
  - Want to remove the mutated allele from the population **over time**
    - DO NOT BLINDLY REMOVE CARRIERS FROM THE BREEDING POPULATION!

Breeding Decisions for LPN1

Leonberger-Specific: LPN2

- Peripheral neuropathy
- Affected dogs are, on average, aged 6 years, but may show signs as young as 1 year
  - May never show signs
- Slowly-progressive neurological signs
  - Gait abnormality
  - Laryngeal paralysis

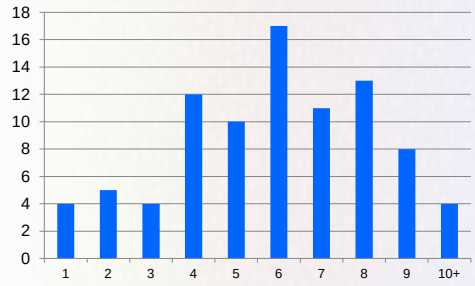
Leonberger-Specific: LPN2

- Mutation Test
  - 2 bp deletion in a "Connexin" gene
    - Connexin gene mutations associated with Charcot-Marie-Tooth disease in humans
- Dominant inheritance with incomplete penetrance
  - All dogs having 1 or 2 copies of the LPN2 mutation ARE GENETICALLY AFFECTED!
  - By 8 years of age, ~65% are showing clinical signs
  - 75-80% will show clinical signs within their lifetime

LPN2 Age of Onset

88 Dogs

Avg. Age of Onset 6 yrs.



LPN2 and Breeding

- The average age of onset is well after the average at which a dog is bred
- Want to prevent affected puppies
- Dogs with even one of the mutated alleles may develop signs of the disease
  - LPN2 affected dogs should not be bred!
  - Only ~6% of Leos have the LPN2 mutation

LPN1 & LPN2

Current Numbers

UMN & Bern

- LPN1
  - NN – 5,958 (85.9%)
  - DN – 913 (13.16%)
  - DD – 65 (0.94%)
- LPN2
  - NN – 6,482 (93.52%)
  - DN – 430 (6.2%)
  - DD – 19 (0.27%)

Polyneuropathy Cases

- Explained by Mutations (~40%)
  - LPN1 – 51 (14.4%)
  - LPN2 – 92 (25.9%)
- Unexplained - 212 (59.7%)
- Total – 355 cases

LEMP

- Leukoencephalomyelopathy
- Degeneration of central nervous system
  - Abnormalities observed on MRI of spinal cord
- Clinical signs: trouble maintaining balance, ataxic (stumbling) gait, some diminished reflexes, may become non-ambulatory
- Age of onset
  - Confirmed cases 1-6 yrs.

LEMP

- We have mapped LEMP to canine chromosome 18
- Currently evaluating genes for the causative mutation
- No genetic test available yet…
- Mode of Inheritance
  - Most likely
    - Autosomal recessive

http://djoeka.net/satoe/

Other Considerations

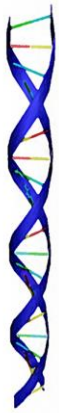
- Life can be complicated…
  - …there ARE pedigrees that have LPN1 AND LPN2 AND LEMP in them!
- There also appears to be a third form of LPN (e.g., LPN3) out there…
- Clear by parentage?
  - The ONLY way to know a dog's true status is by testing
    - Accidental breedings
    - Unscrupulous individuals

OFA/CHIC Clear By Parentage Policy

1. The sire and dam have to be DNA tested "Clear"
2. The sire and dam's DNA disease test results have to be OFA registered
3. All three (sire/dam/offspring) have to be DNA identity profiled and parentage verified

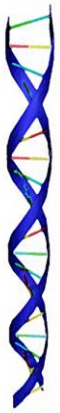
- Then….
- [O]nly first generation offspring will be cleared

http://www.ofa.org/cbp.html



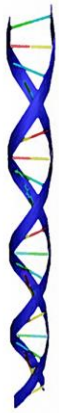
How Parentage Testing Works
a.k.a. "Who's your daddy?

- Typically uses microsatellites
  - Short repeats of ~2-7 nucleotides
    TTTATTTATTTATTTATTTA
    - Number of repeats varies in a population, so, lots of alleles
    - Inherited just like genes
  - Need to test an adequate number
- Alleles in offspring should match either dam or sire
- Parentage testing can ***exclude*** a parent with 100% confidence
  - Can only ***support*** that a parent is a parent



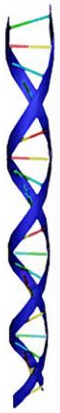
Parentage Testing

- It is possible in dogs (and cats) to have > one sire per litter
  - Parentage testing can help sort this out
- AKC does allow two sires for one litter



New Technologies: SNP Arrays

- SNP arrays
  - SNP = single nucleotide polymorphism
  - One-letter changes
  - Millions of them in every mammalian genome
  - Can test tens, now hundreds, of thousands of them at once
- Dog SNP arrays
  - 1st generation: 22,000
  - 2nd generation: 173,000 (COST: down to $99/each!)
  - 3rd generation: 600,000-1.2million (COST: $225+/each)
    - Coming soon!
- Once data is obtained for a dog on a SNP array, it can be used in any future study!
  - Another reason to keep those health updates coming to us!



New Technologies: NGS

- NGS = Next-generation Sequencing
  - AKA: Whole-genome sequencing (WGS)
- Literally sequences the entire genome of a dog
  - Cost/dog is around $2500
  - 27.5 Gb data/dog, covers genome about 12X
  - Analysis takes a while…
- Again, once a dog is sequenced, that information can be used in other studies!

Other New Technologies

- Exome Sequencing
  - Like NGS, but only sequences the parts of the genome that directly become proteins (ignoring the "in between" stuff)
- RNA Sequencing
  - Like NGS, but only sequences the RNA in the cell
    - RNA is the copies of the DNA that ultimately become proteins (which do the actual work)

New Technologies

- These new technologies have already benefitted Leonbergers!
- LPN1 was found using the 2nd generation SNP arrays
- LPN2 - 2nd generation SNP arrays + NGS
- LEMP - 2nd generation SNP arrays + NGS (ongoing)
  - We have WGS on 41 Leonbergers!

wikipedia.org

Thank You!!!

- For having us here to talk with you!
- For all the funding you have provided to date for our research
  - It's been a very mutually beneficial relationship!

Any Questions?

Kari:
eken0003@umn.edu
Katie:
minork@umn.edu
LPN Info:
lpninfo@umn.edu

dreamstime.com