Neuronal Degeneration in Great Pyrenees: Genetics and Breeding Decisions

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About Me
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• Leonberger Polyneuropathy I (LPN1)
  – Saint Bernards, too
• Leonberger Polyneuripathy II (LPN2)
• Progressive Retinal Atrophy
  – Papillons
Genetics Review
Review Terms

• Gene: unit of inheritance, transferred from a parent to offspring, determines some characteristic of the offspring
  – A specific sequence of “letters” (A,G,C,T), a small sub-set of a chromosome, which “codes” for a protein (protein does some function)
• Allele: different versions of a gene
  – Change in the letters
  – Can be “fatal” change (protein not produced, which may or may not be fatal to the individual)
  – Can be minor change that alters coat color
• Mammals typically have TWO alleles for each gene (one from mother one from father)
Review Terms

• Homozygous: both alleles are the same for a given gene
• Heterozygous: having different alleles for a given gene (sometimes called a carrier)

AA  Aa  aa
Review Terms

• Dominant (mutation): Requires only one allele of a given gene to determine a characteristic

• Recessive (mutation): Must have both alleles the same to be expressed
  – Carriers of recessive alleles (one normal and one mutated allele) are normal
Modes of Inheritance

• Single-gene
  – Recessive
    • Recessive inherited diseases are the most common type of single-gene disorders in dogs
      – Usually from inbreeding
    • Animal must inherit two copies of the mutant allele, one from each parent, to have the altered trait/characteristic
      – Animal with one copy of the mutant allele (a carrier) or an animal with two copies of the normal (non-mutant) allele has normal trait/characteristic
Modes of Inheritance

• Single-gene
  – Dominant
    • Only one copy of the mutant allele, which can come from the sire or the dam, is needed to make the altered trait/characteristic
      – Usually “de novo” (brand new) mutations
    • Animal only has to inherit one copy of the mutant allele, from either parent, to have the altered trait/characteristic
Recessive Trait

- Not easy to identify
  - Parents are not affected
- Several thousand recessive traits have been observed in humans
  - Dogs are getting close 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
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, 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
- Dog genome sequence was published in 2005
Our Work

• We work with many different breeds in our research lab
• We use the strictest confidentiality
  – All identifying information is used with owner permission
Great Pyrenees Neuronal Degeneration (NDG)
How it Began..

- Two siblings, started showing some neurological signs
- Toxin?
- Trauma?
- Genetic?

(Came in for LPN1 and LPN2 testing)
(Normal for these)
Clinical Signs

• Neurologic signs
  – Begins mildly: slipping/sliding/difficulty maneuvering on slippery surfaces
  – Abnormal gait: dog may seem clumsy, uncoordinated, or weak
  – Most pronounced in hind limbs, and it is symmetrical
  – These signs slowly progress and worsen over time

• Age-of-onset: very young
  – Four months or possibly even younger
  – Progression is over years

• VIDEOS
Clinical Signs, Continued

- Eventually: wide-based stance, can’t negotiate stairs, and generalized loss of control of body movement coordination
  - Especially when moving fast/quickly
  - May experience falling
  - Condition is non-painful (although falling is!)
  - Will progress to non-weight-bearing/non-ambulatory
Necropsy

• Pathological changes in the neurological system
  – Central nervous system: changes in the brain and spinal cord
  – Peripheral nervous system: changes in the peripheral nerves
Histopathology

Brightly eosinophilic structures (necrotic neurons) in the medial geniculate nucleus.

No Purkinje cells noted in an entire folium of the cerebellum.
Clinical Signs (Doctor Version)

- Widespread central nervous system degeneration: cerebellar ataxia and spasticity
- Also peripheral neuropathy
- Demyelinating disorder with secondary nerve fiber loss
- Chose to call it: NEURONAL DEGENERATION (NDG)
Investigation!

- Examination of pedigrees showed a recessive mode of inheritance
  - Affected dogs were relatively closely related
- Breeders and owners mobilized
  - Within weeks, we had DNA samples (blood) in the lab from 6 cases and ~40 related dogs
- Conduct GWAS
GWAS

• Genome-wide Association Study
• Whole-genome approach using dense markers or “tags”
  – Detect regions of chromosomes where the allele frequencies are different in cases versus controls
    • All dogs are tested for all the markers simultaneously
  – If the mutation arose on a founder several generations ago, then the cases will share the region near that mutation
  – The GWAS doesn’t detect the mutation – only a region to look at more closely
Linkage Disequilibrium

- Association mapping works because of LD
GWAS

- 6 cases and 26 controls – all tested simultaneously
- Illumina Canine High Density SNP Arrays
  - 173,000 markers tested on each dog
  - SNP = Single Nucleotide Polymorphism (DNA sequence variation with a single letter difference) – used as a marker
    - Do alleles of a SNP segregate with disease?
- Statistical analysis
GWAS - Findings

– “Homozygosity mapping” approach
  • Where are all the cases the same?
– Identified 3.3 Mb region on CFA25
  • 28 of the SNP markers were in perfect LD
  • All cases were homozygous
  • All controls were heterozygous or homozygous for the opposite alleles

• ....~200 genes in the region!
Excellent positional and functional candidate gene: **SACS**

SACS is involved in regulating mitochondrial dynamics
- Expressed ubiquitously in the CNS
- Function not entirely clear

Sanger sequencing
- 4 bp deletion in exon 9
  - Causes frameshift and truncates ~1000 bases of the mRNA
ARSACS

• **SACS** is mutated in human syndrome ARSACS (autosomal recessive spastic ataxia of Charlevoix-Saguenay) (Quebec, Canada)
  – Characterized by progressive cerebellar ataxia and peripheral neuropathy
  – Muscle spasticity and muscle wasting
  – Speech difficulties
  – Eye changes (hypermyelination)
  – Age-of-onset: 1 yr to 1.5 yrs
  – Wheelchair by 30s-40s
NDG: Why were we successful?

• Genetic test now available for Great Pyrenees (tests for 4bp deletion in *SACS*)

• Successful due to quick action of breeders to work with us

• Successful because of available genomic tools for dogs and our lab’s expertise in using them

• A little bit of luck
Total Tested To-Date

- 535 Great Pyrenees tested
  - 8 affected (homozygous for mutant allele)
  - 67 carriers (heterozygous)
  - 460 normal (homozygous for normal allele)
- 12.5% carrier rate
Breeding Decisions

• Recessive mutations are everywhere
  – We all have them lurking in our genomes (on average, every mammal has at least a dozen harmful recessive mutations hidden in their genome)
  – The NDG Pyr project is a perfect model for how to cope with these situations when they arise (and they will, in any breed of dog)
    • Transparency is key
    • Quick mobilization to get samples to researchers

• Remember there are ~20,000 genes
  – SACS is one of them
    • And we can TEST for this one!

• What to do with carriers?!
Breeding Decisions for NDG

Breeding two clear dogs

Breeding a clear dog to an affected dog

Breeding a clear dog to a carrier dog

Breeding a carrier dog to an affected dog

Breeding two carrier dogs

Breeding two affected dogs
Breeding Decisions

- On the previous slide, you should avoid crosses that produce “red” (affected) puppies
- The other crosses are okay!
- If you have a fantastic dog, terrific qualities, but happens to be a carrier for NDG, go ahead and use him/her!
  - Just breed to a clear dog
  - Test the litter so you know which pups are clear and which are carriers
  - Over time, replace your breeding stock with clear dogs
- REMOVING ALL NDG CARRIERS IMMEDIATELY FROM THE GENE POOL WILL JUST CREATE NEW PROBLEMS
  - Why throw out the 19,000+ other terrific genes in a dog because it happens to carry on mutation that we can test for?!?!
    - That’s throwing out the baby with the bath water
  - Remember, there are recessive mutations in there – in EVERY dog! – that we CANNOT test for
  - Use the test wisely and continue breeding great dogs
Carriers

• Consider ElC for a moment:
  – Carrier rate is ~30% in field trial Labrador Retriever lines
  – Remove carriers from the gene pool?
    • This would be catastrophic! It is NOT a good idea to remove 30% of the gene pool! It will just create new problems!

• NDG in Great Pyrenees
  – Carrier rate is 12.5%
    • Same here – you don’t want to throw out 12% of your gene pool because of one gene (that you can test for!)
    – **TEST and REPLACE (SLOWLY)**
      • Use carriers as you would in your breeding (use great dogs), and just make sure you test the offspring
        – Slowly, over several generations, you can breed out the NDG mutation

• **The healthiest breeds consider the entire breed population!**
Other Considerations

• Posting NDG results to OFA
  – $15 cost
  – OFA posts affected results for free
  – Most success obtained with transparency

• Clear by parentage?
  – The ONLY way to know a dog’s true status is by testing
    • Accidental breedings
    • Unscrupulous individuals
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
UMN Canine Genetics Laboratory Website

Our interdisciplinary team is working to understand the cellular, molecular, and genetic basis of inherited neuromuscular, neurological, and metabolic disorders of companion animal species.

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Canine Genetic Testing
Canine Genetic Research
An inherited neurological disease, called "Neuronal Degeneration" or NDG, has been reported in Great Pyrenees dogs. The age-of-onset of this disease is very young, well before an affected dog’s first birthday, but begins quite mildly. Initial signs include slipping, sliding, and difficulty maneuvering on smooth surfaces. The gait is abnormal - the dog may seem weak, clumsy, or uncoordinated. Over time, these problems progress and worsen. The abnormalities are most pronounced in the hind limbs, and both sides of the body tend to be affected symmetrically. Eventually, affected dogs display a wide-base stance, become unable to negotiate stairs, have a generalized loss of control and coordination over body movements, and may experience intermittent falling. The condition itself is non-painful, although stumbling and falling can obviously cause pain and traumatic injuries.

Necropsy of the neurological system reveals pathological changes throughout (in the brain, spinal cord, and peripheral nerves). The disease will continue to progress until the dog may not be able to support its own weight or walk on its own.

Research carried out at the University of Minnesota indicates that this disease is inherited in an autosomal recessive manner. We have identified a mutation within a disease-associated gene, and all affected dogs have two copies of this mutation. We are now offering a genetic test which allows owners to determine their dog’s status for this mutation in order to diagnose affected dogs and to guide future breeding decisions.
Future Work

• Once data is obtained for a dog on a SNP array, it can be used in any future study!
  – May require health updates

• Dwarfism?
Thank You!!!

• For having us here to talk with you!
• For all the funding you have provided to date for the research
  – It’s been a very mutually beneficial relationship!
ANY QUESTIONS?

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