Molecular Genetics

Simon Petersen-Jones
Professor, Comparative Ophthalmology
Dept. Small Animal Clinical Sciences
Michigan State University
What’s your diagnosis?
Mendelian Inheritance

• Mendel’s laws apply to genes on separate chromosomes
• The simplest genetic characteristics depend on the genotype at a single locus
• Modes of inheritance:
  – autosomal recessive
  – autosomal dominant
  – X-linked recessive
  – X-linked dominant
The Work of Gregor Mendel

- Mendel crossed pea plants with different characteristics and observed the resulting physical appearance (phenotype)
  - round peas vs wrinkled peas
  - yellow peas vs green peas
  - gray seed coat vs white seed coat
  - tall plants vs short plants
The Work of Gregor Mendel

• Mendel presented his findings in 1865 to a natural history society
• Published “Experiments with plant hybrids” in 1866
• Frustrated by lack of interest in results became an administrator
What We Learned from Mendel

• There is a specific pair of genes for each trait
  – the genotype for a given trait is specified by a pair of genes
  – some loci may have more than one allele

• During formation of gametes the gene pair for a trait segregates equally
  – one gamete only receives one of a pair of genes
  – Mendel’s law of equal segregation (first principle)
What We Learned from Mendel

• A gene has two forms - alleles (e.g. $A$ and $a$)
  – genotype $aa$ (homozygous for $a$) express a recessive phenotype
  – genotypes $AA$ or $Aa$ express a dominant phenotype
What We Learned from Mendel

• During formation of gametes the segregation of the gene pair for any one trait is independent of the segregation of other gene pairs
  – if heterozygous for 2 traits \((AaBb)\) gametes \(AB, Ab, aB, ab\) produced with equal probability
  – **law of independent assortment** (second principle)
  – applies to characteristics that are not genetically linked
Punnett Squares to Follow
Transmission of a Trait

Female Gametes

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1 : 2 : 1
Punnett Squares to Follow
Transmission of a Trait

<table>
<thead>
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1 : 1
Autosomal Dominant Inheritance
Autosomal Recessive Inheritance
X-linked Recessive Inheritance
X-linked Dominant Inheritance
Ocular Melanosis
Stage IV. Ocular Melanosis
Cairn Terrier – Ocular Melanosis
Hardy-Weinberg Equilibrium

\[ p^2 + 2pq + q^2 = 1 \]

\[ P = \text{frequency of dominant allele} \]
\[ q = \text{frequency of recessive allele} \]

- For a breed with an autosomal recessive disease with an incidence of disease of 10% what percentage of the population will be carriers?
Hardy-Weinberg Equilibrium

\[ p^2 + 2pq + q^2 = 1 \]

For a breed with an autosomal recessive disease with an incidence of disease of 10% what percentage of the population will be carriers?

- \( p^2 \) = homozygous normal = 46.8%
- \( 2pq \) = heterozygous carriers = 43.2%
- \( q^2 \) = homozygous affected = 10%
- \( \therefore q^2 = 0.1 \). \( \therefore q = 0.316 \)
- \( p = 1-q \). \( \therefore p = 1 - 0.316 = 0.684 \)
- \( 2pq = 2(0.684 \times 0.316) = 0.432 \)
- Carrier rate = 43.2%
Exceptions to Hardy-Weinberg Equilibrium

• H-W Equilibrium means that proportion of genotypes remain the same from generation to generation. This only occurs if:
  – population is large
  – members mate at random
  – no migration
  – no mutation
  – no natural selection
Apparent Alteration to Mendelian Ratios

Mendel’s laws apply to the underlying genotype - not always reflected in phenotype

• Incomplete dominance
  – different alleles that are expressed in heterozygote are co-dominant

• Epistasis
  – the masking of the effect of one gene by another gene

• Incomplete penetrance
  – some individuals do not express the phenotype
Apparent Alteration to Mendelian Ratios

Mendel’s laws apply to the underlying genotype - not always reflected in phenotype

- **Pleiotrophy**
  - a Mendelian disorder resulting in different manifestations

- **Phenocopy**
  - a noninherited environmentally caused trait that mimics one that is inherited

- **Genetic heterogeneity**
  - a characteristic that can be caused by different genes
Incomplete Dominance

different alleles that are expressed in heterozygote are co-dominant

• ABO blood types - determined by 2 alleles of the I gene (alleles are $I^A$, $I^B$ or $i$)
  – type A blood group = $I^A I^A$ or $I^A i$
  – type B blood group = $I^B I^B$ or $I^B i$
  – type AB blood group = $I^A I^B$
  – type O blood group = $ii$
Epistasis

the masking of the effect of one gene by another gene

• *H* gene allows for expression of *I* gene (A & B antigens on RBCs)
  – *HH* or *Hh* allows normal expression of A & B antigens
  – *hh* does not allow for expression of A & B antigens - antigen test shows all genotypes to be O phenotype (Bombay phenotype)
Incomplete penetrance

some individuals do not express the phenotype

- Degree of penetrance described as a percentage
- Dominant with incomplete penetrance suggested for posterior polar cataract in Labrador retriever
Pleiotrophy

a Mendelian disorder resulting in different manifestations

- Oculoskeletal dysplasia
  - retinal dysplasia
  - skeletal dysplasia
- Marfan syndrome (fibrillin-1 mutation)
  - multiple ocular abnormalities
  - abnormalities of several other systems
Phenocopy

a noninherited environmentally caused trait that mimics one that is inherited

- Noninherited disease that looks like a known hereditary disease
  - SARDs & PRA

- Noninherited disease that appears to follow a pattern of inheritance
  - retinopathy in performance dogs
Genetic heterogeneity

a characteristic that can be caused by different genes

- Progressive retinal atrophy
- Retinitis pigmentosa
- Oculoskeletal dysplasia
Lethal Allele Combinations

- **Mexican Hairless Dog**
  - $H$ is the allele for hairlessness
  - $hh = \text{hairy}$
  - $HH = \text{lethal}$
  - $Hh = \text{hairless}$
Non-Mendelian inheritance - mitochondrial

- Mitochondrial DNA differs from nuclear DNA
  - several copies per mitochondria
  - no cross over during reproduction
  - faster mutation rate
  - not wrapped in histone proteins
  - no introns
  - lack of repetitive elements

- Synthesize rRNA, tRNA & a few proteins of oxidative phosphorylation system
Non-Mendelian inheritance - mitochondrial

- Mitochondria DNA very similar to α purple bacteria
  - resulted from endocytosis of purple bacterium by anaerobic eukaryotic precursor cell 1.5 billion years ago
  - advantageous in developing oxygen atmosphere to have oxidative phosphorylation system
- Mitochondrial genome smaller than bacterial genome (e.g. *E. Coli* – 4.6Mb; mammalian mitochondria < 20 kb)
  - transferred genetic material to nuclear genome
Non-Mendelian inheritance - mitochondrial

- Human mitochondrial DNA - 16,569 bases, 37 genes
- Canine mitochondrial DNA - 16,728 bases
- Mitochondria are maternally inherited (via ovum)
- Mitochondrial diseases show maternal mode of inheritance
Non-Mendelian inheritance - mitochondrial

• Heteroplasmcy - mitochondrial gene mutation present in only some of the mitochondria
  – expressivity varies between siblings
  – severity depends on which tissues have the mitochondria with the mutation
  – signs of disease often do not appear until adulthood (several cell divisions until enough mutant mitochondria accumulate)
  – e.g. Leber Hereditary Optic Neuropathy (starts as loss of vision in early adulthood)
Non-Mendelian inheritance - imprinting

• Differential expression of the two alleles of a gene dependent on parental origin
  – uniparental expression
  – maternal allele ≠ paternal allele

• Prader-Willi syndrome
  – deletion of part of paternal 15q12
  – mental retardation, hypotonia, obesity, hypogenitalia

• Angelman syndrome
  – deletion of part of maternal 15q12
  – mental retardation, growth retardation, hyperactivity, inappropriate laughter
Mutations

• Germline mutations - inherited
• Failure of DNA repair enzymes to correct mistake
• If alter protein coding region or regulatory region may result in a phenotype
  – mutations may be deleterious
  – may confer an advantage - evolution
    • e.g. mutation causing sickle-cell anemia gives heterozygote an advantage
Mutations

- Single-base pair
  - transition
    - purine to purine $A \rightarrow G$ or $G \rightarrow A$
    - pyrimidine to pyrimidine $C \rightarrow T$ or $T \rightarrow C$
  - transversion
    - purine to pyrimidine or vice versa
  - missense - alter amino acid coded
  - nonsense - introduce premature stop codon

- Insertions
  - SINEs (short interspersed element). SINE insertion found to be responsible for merle phenotype

- Deletions (in frame and not in frame)
Mutations

- Alteration in number of repeats
  - expansion in number of triplet repeats can cause disease in humans
  - such diseases exhibit “anticipation” i.e. phenotype worsens in successive generations
    - myotonic dystrophy
    - Huntington disease
  - Epilepsy in miniature wirehaired dachshunds (Epm2b gene 19 to 16 copies of a 12 bp sequence – Lohi et al Science 2005)
How Mutations Alter Function

• *Null* mutation (no functional gene product), often recessive disease
  – mRNA broken down
  – protein if produced lacks important domain

• Increased activity
  – constitutively active

• Dominant negative effect
  – e.g. binds receptor but is inactive

• Altered protein structure
  – misfolding affects function or alters trafficking resulting in abnormal accumulation
Rhodopsin Mutations

- **Autosomal Dominant RP**
  - folding defect
  - transport defect
  - constitutively active

- **Autosomal Dominant Congenital Stationary Night Blindness**
  - constitutively active

- **Autosomal Recessive RP**
Chromosomal disorders

- Deletions of portions
- Translocations
- Repetition of chromosomes
Linkage of Genetic Traits
Separation of Genetic Material During Meiosis

- Characteristics on separate chromosomes segregate independently
  - law of independent assortment (Mendel’s second principle)
  - Not followed for linked characteristics
- “Chunks” of chromosomes are inherited together (linked) – Region of Linkage Disequilibrium (LD)
Linkage

• The closer traits are on a chromosome the less likely they are to become separated during meiosis

• A crossover between linked genes is called a recombination
Nonrecombination of linked genes

Diagram showing the process of nonrecombination in meiosis. Initially, replicated homologous chromosomes are shown. During Meiosis I, homologous parts remain together, and during Meiosis II, centromeres separate to form parental gametes.
Recombination of linked genes
• Identity by descent
  – All have the same mutation from a founder animal
  – Region identical by descent around the causal mutation
    • Length depends on number of meioses
Genetic Distance (x)

• Genetic distance (x) is measured in Morgans
  – it is the average number of crossover points between 2 loci on a gamete
  – 1% recombination = 1cM apart

• Physical distance is measured in basepairs
• Genetic distance ≠ Physical distance
Lod Score

• Logarithm of the odds (Z)

• logarithm of the odds that two loci are linked (with recombination fraction \( \theta \))

• Recombination fraction \( \theta \), is proportion of recombinations out of the total of recombinations and nonrecombinations
  – As only 2 out of 4 gametes are affected by a crossover maximum value for \( \theta =0.5 \) (unlinked)
  – With close linkage \( \theta \) tends to 0.

• Lod score of 3 or more is considered significant
Characteristics for Disease Markers

- DNA markers that are closely linked to disease locus
- DNA variations giving rise to detectable characteristics
  - Coat color, eye color, blood group, tissue type
  - Can detect externally or with a simple test
- DNA variations that are not expressed
  - Can detect by examining the DNA
DNA Variations used as Genetic Markers

- Variations in DNA sequence established in a population – polymorphisms
  - Do not cause disease
  - Inherited in a Mendelian fashion
  - Can be identified in an individual (genotyped for the variation)
Mapping Genetic Characteristics

- Genetic markers are required across the genome for mapping genes
- Genetic markers
  - minisatellites
  - restriction fragment polymorphisms
  - microsatellites
  - SINEs (short interspersed element) – “jumping genes” retrotransposons
  - single nucleotide polymorphisms (SNPs)
Microsatellites

• Short arrays of tandem repeats (1-4bp)
• CA repeats very common
  – 0.5% of the genome
• CT repeats common
  – 0.2% of the genome
• tri- & tetra- nucleotide repeats rarer
  – trinucleotide repeats expansion can cause disease
• May have several alleles
Microsatellite
Single Nucleotide Polymorphisms

- Polymorphism of one nucleotide
- May occur up to every 500 basepairs
- Only two alleles
- Spread across entire genome
- Useful for mapping as numerous and easy to type
- Dog SNPs available – Broad Institute at MIT.
  http://www.broad.mit.edu/mammals/dog/
Single Nucleotide Polymorphism
Hereditary Eye Disease

- Conformational abnormalities – likely complex traits
  - Lids: entropion, ectropion
- Cilia abnormalities?
  - Distichia, ectopic cilia
- Corneal dystrophies
  - Crystalline stromal dystrophies
  - Endothelial – Fuchs Endothelial Dystrophy
- Glaucoma
  - Primary
    • ADAMTS10 (beagle POAG)
  - Ocular Melanosis – mapped location
- Uvea
  - Pigmentary uveitis
  - PPM
Hereditary Eye Disease

- Cataract
  - *HSF4* mutations
- Lens luxation
  - *ADAMTS17*
- Syndromic
  - Oculoskeletal dysplasia (dwarfism with retinal dysplasia: *drd1, drd2*)
    - *Col9A2, Col9A3*
    - Ocular defects in “White” Dobermans
      - *SLC45A2*
- Entire Globe
  - Total retinal dysplasia
  - Microphthalmia
  - Merle defects
- Retina/choroid
Hereditary Retinal/Choroidal Disease

- Collie Eye Anomaly
  - Intrinsic DNA change in *NEHJ1*
- Retinal Dysplasia
- Canine Multifocal Retinopathy (*cmr1, cmr2, cmr3*)
  - *BEST1*
- Progressive Retinal Atrophy
  - Many genes
- Cone Rod Dystrophies
  - Several genes
- Achromatopsia (day blindness)
  - *CNGB3*
- Retinal Dystrophy of Briards (congenital stationary night blindness)
  - *RPE65*
- Congenital stationary night blindness – Appaloosa
  - *TRMP1*
Identifying the cause of a genetic disease

Looking for needle in a haystack (1 in 2.4 billion)

- Functional cloning
  - altered function detected
- Candidate gene
- Whole Genome Approaches
  - Identify disease locus
    - Linkage mapping
      - Good pedigree with necessary power to map locus
    - Genome Wide Association Study (GWAS)
      - Association between disease status and polymorphic markers (SNPs)
  - Heterozygosity mapping
    - Positional candidate genes?
FUNCTIONAL CLONING
PRA in Irish Setters -<em>rcdl</em>

- Night blind at 6 weeks of age
- Electroretinogram never develops normally
- Photoreceptors do not form normally
  - rods non-functional
  - cones functional but slowly degenerate
- Blindness between 1 and as late as 6 years
History of Detection of *rcdl* Gene Mutation

- PRA in Irish setters exported to USA
- 1970s – Aguirre et al describes rod-cone dysplasia
  - ERG
  - histopath
  - biochemical - early accumulation of cGMP, lack of PDE activity

\[ \text{rhodopsin} \rightarrow \text{transducin} \rightarrow \text{cGMP} \rightarrow \text{phosphodiesterase} \rightarrow 5' \text{GMP} \]
History of Detection of \textit{rcd1} Gene Mutation

- 1992 - Levels of transduction gene mRNAs measured - found cGMP phosphodiesterase beta reduced prior to others
  – Farber, Danciger & Aguirre
- 1993 - mutation found in cGMP PDE beta gene
  – Suber, Pittler et al
- 1993 - development of first DNA test for PRA
  – Clements et al
rcdl-PRA in Irish Setters

Mutation in cGMP phosphodiesterase beta subunit

Nucleotide 2420 G → A

Normal TGG = tryptophan
Mutant TAG = stop codon

Exon 21
Candidate Gene Approach
Candidate Gene Approach

• Detailed analysis of disease
  – What gene could cause this phenotype
  – Genes known to cause this phenotype in humans, mice, other breeds

• Some disease may have a large number of candidates e.g. PRA
  – visual transduction genes
  – retinal structural genes
  – genes expressed elsewhere that are functional in the retina
Candidate Gene Approach

- Select candidate
- Sequence in normal and affected individual
- Rapid screening method for alterations in sequence
- Identify polymorphisms in candidate gene and see if co-segregates with disease status
  - if locus linked to disease screen gene sequence or use rapid screening method
Mapping of Recessive Genetic Defects

• Identity by descent
  – All have the same mutation from a founder animal
  – Region identical by descent around the causal mutation
BASIC SCIENCE

Identification of mutations in HSF4 in dogs of three different breeds with hereditary cataracts

Cathryn S. Mellersh,* Louise Pettitt,* Oliver P. Forman,* Mark Vaudin* and Keith C. Barnett†

*Centre for Preventive Medicine and †Comparative Ophthalmology Unit, Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU, UK
Identification of Cataract Gene

• Candidate gene combined with linkage
• Selected 20 candidate genes
• Selected microsatellites linked to each candidate gene
• Microsatellites linked to HSF4 “associated” with cataract in Staffordshire Bull Terrier
• Sequenced gene – identified mutation
• Looked at gene in other breeds

Hereditary Cataracts

• Heat-shock transcription factor 4 (HSF4)
  – Mutations cause cataract in children & mice
  – Staffordshire Bull Terrier & Boston Terrier – 1bp insertion = frame shift, 27 altered amino acids then a premature stop codon
  – Australian Shepherd – 1 bp deletion = frame shift, 86 altered amino acids then a premature stop codon

Whole Genome Study
Positional Cloning

- Establish tight linkage to disease locus
  - Microsatellites (need good pedigree)
  - SNPs – can use many more loci and look for “association”
- Eventually map disease locus between 2 markers (confidence interval)
  - May still be several genes in interval
- Examine genome database for known genes in that region (beware not all genes identified)
- Select “Positional Candidate Gene” - sequence
Identification of PRCD locus

- Mapped to canine chromosome 9
- Examined all known genes – nothing
Progressive Rod Cone Degeneration

- thymidine kinase 1
- galactokinase 1, *prcd*
- atrial myosin alkali light chain 1
- Retinoic acid receptor α
- neurofibromin

Canine chromosome 9
Identification of PRCD locus

- Using DNA from 14 PRCD breeds narrowed interval for PRCD using linkage disequilibrium
  - From 6.4Mb to 106kb (3 known genes)
- Examined retinal cDNA library for unknown genes mapped to confidence interval
- Eventually identified novel gene (found expressed in retinal cDNA) with a mutation
  - Known as PRCD

Identification of *PRCD* Mutation

- *PRCD* gene mutation
  - Mutation codon 2 TGC $\rightarrow$ TAC
  - Cysteine $\rightarrow$ Tyrosine
  - Alters protein distribution when expressed in cell culture
PRCD Gene

- Codes a 54 AA protein
  - Transmembrane protein? Function?
- Expressed retina and RPE/Choroid
  - Lower levels in some other tissues (RT-PCR)
- Laser capture microscopy – RT-PCR
  - Showed similar levels in ganglion cells, photoreceptors & RPE

Human \textit{PRCD}

- 1863 RP patients screened
- Patient from Pakistan
  - Exact same mutation as \textit{PRCD} dogs!!

PRCD Breeds

- Am & Eng Cocker Spaniel
- American Eskimo Dog
- Australian Cattle Dog
- Australian Shepherd
- Australian Stumpy Tail Cattle Dog
- Chesapeake Bay Retriever
- Chinese Crested (also have non-prcd PRA)
- Cockapoos
- Entlebucher Sennenhund (Swiss Mountain Dog)
- Finnish Lapphund
- Golden Retriever
- Kuvasz
- Labradoodles
- Labrador Retriever
- Lapponian Herders
- Miniature & Toy Poodle
- Nova Scotia Duck Tolling Retriever
- Portuguese Water Dog
- Spanish Water Dogs
- Swedish Lapphunds
- Others

www.optigen.com
GENOME WIDE ASSOCIATION STUDY (GWAS)
HOMOZYGOZITY MAPPING
Genome Wide Association Study (GWAS)

- Illumina HD Canine SNP array - 170K SNPs

SNP – single nucleotide polymorphism

- allele 1: TTTGTGCCCTAGTCCCTGGG
- allele 2: TTTGTGCCCCTAGTCCCTGGG

**C/C** homozygous

**C/T** heterozygous

**T/T** homozygous
Illumina Dog SNP Chips

Figure 1: CanineHD BeadChip

The CanineHD Genotyping BeadChip features more than 170,000 evenly-spaced SNPs across the entire dog genome.
GWAS mapping using SNPs

• Look at a number of disease affected dogs (same disease – same gene mutation) and compare with breed matched unaffected dogs

• Statistical association between each SNP and the disease
Primary Lens Luxation

- Mode of inheritance identified
- Microsatellite mapping of locus
- SNP GWAS mapping + Microsatellite fine mapping
Primary Lens Luxation

- Genome-wide mapping with microsatellites
  - 6.3Mb region chromosome 3
Primary Lens Luxation

- GWAS – Illumina SNP20 (22,000 SNPs)
- Jack Russell Terriers
  - 28 cases
  - 20 controls
- Fine Mapping – Chromosome 3 -20MB to 70MB (768 SNPs)
- Miniature Bull Terriers
  - 38 cases
  - 11 controls
- Lancashire Heelers
  - 22 cases
  - 18 controls
Primary Lens Luxation

- Sequencing of positional functional gene
  - Four genes in mapped region
  - ADAMTS17 structurally similar to ADAMTS4L – causes recessive ectopia lentis in man
An ADAMTS17 Splice Donor Site Mutation in Dogs with Primary Lens Luxation

Fabiana H. G. Farias,1 Gary S. Johnson,1 Jeremy F. Taylor,2 Elizabeth Giuliano,3 Martin L. Katz,1,4 Douglas N. Sanders,4 Robert D. Scbnabel,2 Stephanie D. McKay,2 Shabnawaz Khan,1 Puya Gharabkhani,5 Caroline A. O’Leary,5 Louise Pettit,6 Oliver P. Forman,6 Mike Boursnell,6 Bryan McLaughlin,6 Saija Abonen,7,8 Hannes Lobi,7,8 Elena Hernandez-Merino,9 David J. Gould,10 David R. Sargan,9 and Cathryn Mellersh6

IDENTIFICATION OF PRA1 GENE

Paige Winkler – Genetics PhD Student
Kari Ekenstedt DVM PhD. Ass Professor U of Wisconsin, River Falls
Progressive Retinal Atrophy

- Described in over 100 breeds
- Genetically heterogeneous
  - arPRA, adPRA, XLPRA
PRA in Papillons

- First described in 1995
- Variable age of onset
  - 1-12 years of age
- Recessively inherited
- Thought to be a middle-age onset
Dark Adapted ERGs

Puppies at 10 weeks of age
Dark-adapted ERG recordings at -2.4, -1.2, 0.4 log cdS/m²
Papillon Vision Testing

- Outcome measures:
  1. Tunnel choice
  2. Time to exit
- Several light intensities tested
  - Poor performance at lowest intensity

A Novel Method for Objective Vision Testing in Canine Models of Inherited Retinal Disease

*Patricia M. Gearhart, Chris C. Gearhart, and Simon M. Peterson-Jones*
Retinal Characterization

- *In vivo* analysis of affected dogs
  - Optical coherence tomography (OCTs)

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Graph showing progressive loss of photoreceptor cells.
Papillon Phenotyping Conclusions

• Lack of rod function – never normal
  – ERG – no dim light response
  – Vision testing – no dim light vision

• Progressive loss of photoreceptors
  – Ophthalmoscoposcopic signs of retinal thinning
  – OCT measurements of retinal layer thicknesses

• Gradual and progressive loss of cone function
**Genome Wide Association Study (GWAS)**

- Illumina HD Canine SNP array - 170K SNPs
  - 9 affected, 4 obligate carriers and 11 unaffected Papillons

SNP – single nucleotide polymorphism

- **Allele 1:** TTTGTGCCCAGTCCCTGGG
- **Allele 2:** TTTGTGCCCTAGTCCCTGGG

- **C/C** homozygous
- **C/T** heterozygous
- **T/T** homozygous
Genome Wide Association Study

• PLINK analysis for association
  – No significant associations (or nearly significant associations) between disease status and SNP genotyping
• More than one type of PRA in the breed??????
Homozygosity Mapping of Recessive Genetic Defects

• Identity by descent
  – All have the same mutation from a founder animal
  – Region of homozygosity around the causal mutation

autozygosity.org
Homozygosity Mapping

• Homozygosity mapping using a custom sorting program

• 3 affected, 2 obligate carriers and 11 unaffected Papillons
Homozygosity Mapping Results

- 13 regions greater than 1.5 Mb in size
- 4 of these regions harbored 6 candidate genes
- Haplotype analysis + phenotypic comparisons
  - Cngbl1 was the top candidate
# Homozygosity Mapping Results

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Sequencing *Cngbl1*: Exon 26
CNGB1 Expression in Rods

Modified from Kaupp and Siefert, 2002

Pearring et al 2013
Retinal Characterization

- IHC – Frozen retinal sections
Cngb1 Mutation in Papillons

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- **CNGB1** mutation accounts for ~70% of PRA in Papillons
- Locus heterogeneity – at least one more form of PRA in Papillons
Congenital Stationary Night Blindness in the Appaloosa

- Negative ERG waveform (similar to human complete CSNB)

- Lack of b-wave suggests lack of ON bipolar cell function

Congenital Stationary Night Blindness in the Appaloosa

- Appaloosa coat spotting caused by single incompletely dominant gene (LP)
- LP/LP associated with CSNB — Sandmeyer et al 2007
- LP locus mapped — TRPM1 mapped to same region
  — Bellone et al 2008
- Affected horses ↓↓ TRPM1 levels (retina & skin)
- TRPM1 – TRP channel – down-regulated in metastatic melanoma (melastatin)
- TRPM1/-/- mice no b-wave
- TRPM1 shown to be mGLuR6 coupled ion channel of ON bipolar cells — Morgans et al 2009, Koike et al 2010
Morgans et al. Bioessays 2010