How to make breeding decisions on traits without EBVs

Madeline Zimmermann, M.S.

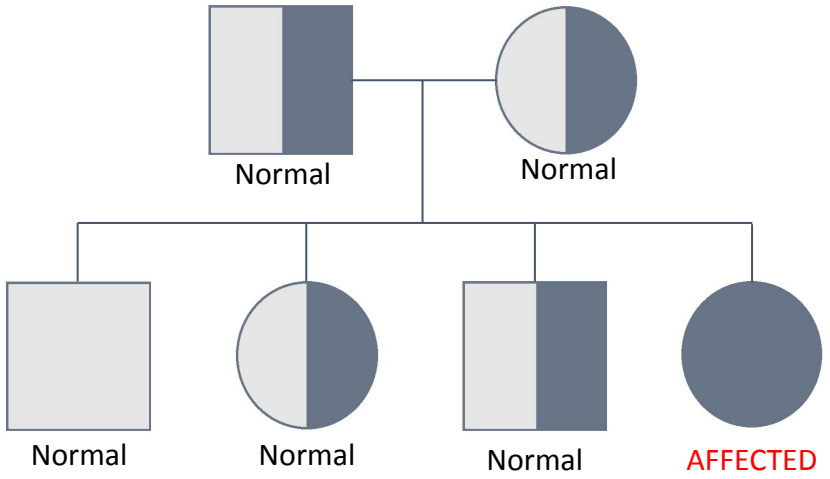
2023 Breeders Workshop

Outline

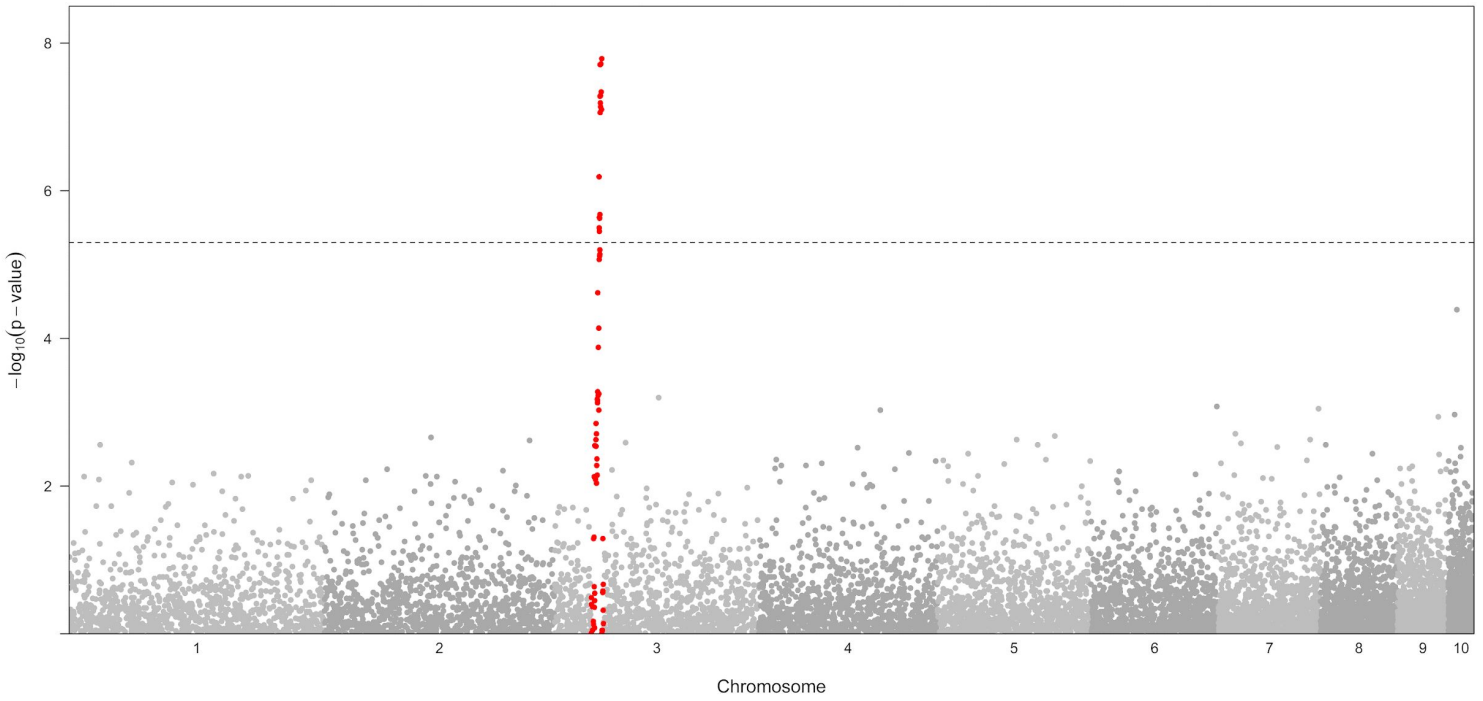
- Background
- A general process for assessing genetic risk when EBVs are unavailable
- Where could this go wrong? (Data considerations)
- Considerations if risk is suggested
- Examples:
  - CLD
  - Head tremors
  - TVD

Many are familiar with **autosomal recessive:**

- Caused by 1 gene
- Need 2 copies of causative variant to show phenotype
- Can do genetic tests, e.g. dog has it or they don't and there's a carrier state that is healthy
- **Rarely applies to traits of interest**

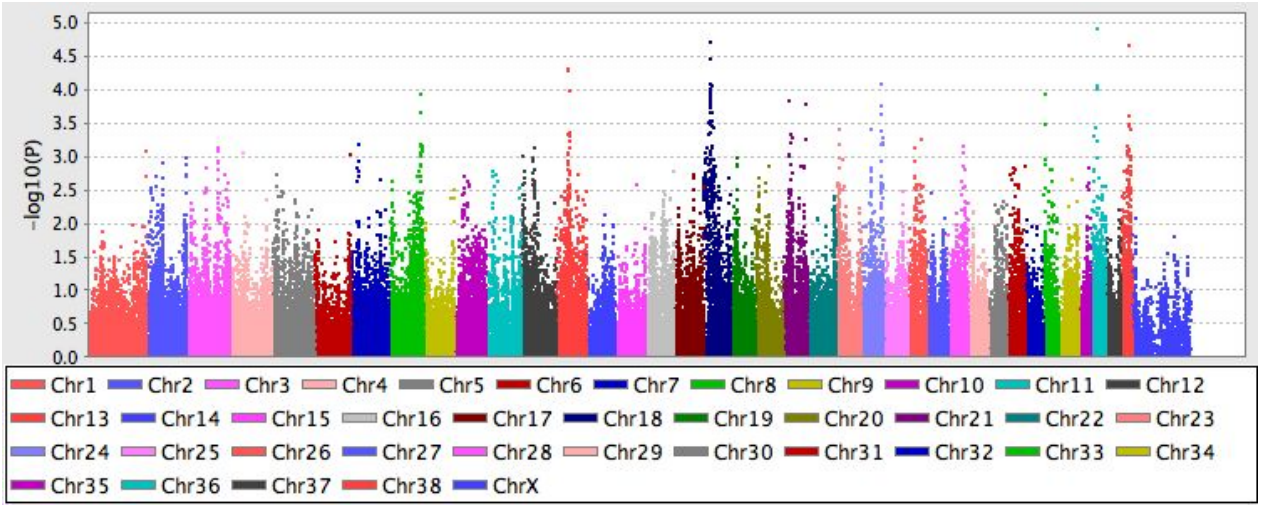


	A	a
a	Aa	aa
a	Aa	aa



Traits of interest are often complicated

- **Polygenic** = many genes of small effect add up to produce the observable phenotype
- Difficult to find reliable genes for testing or understand how genes combine to affect phenotype



From a 2016 GWAS analysis of Mast Cell Tumor cases done by the **Broad Institute** and **Guiding Eyes for the Blind**. No significant associations identified.

Making progress in polygenic traits

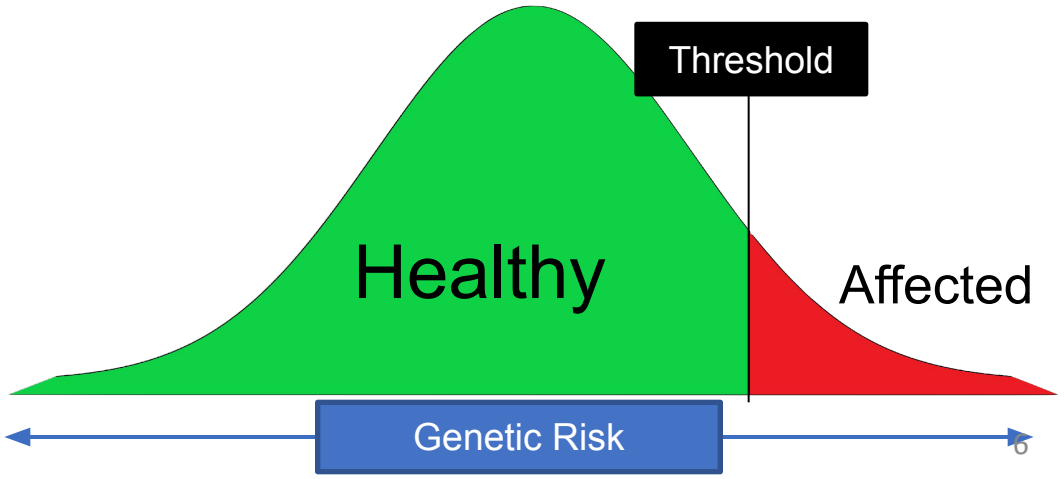
Use EBVs!

- Quantify “how good the dogs’ genes are” based on relevant information available on the dog and all related dogs so you can identify who is higher or lower risk for producing more of the issue.
- Do not need to know specific genes involved or what they do

A General Process for Assessing Genetic Risk When EBVs are Unavailable

Is there an increased incidence when breeding higher risk dogs?

1. Identify affected dogs.
2. Classify other dogs:
    1. “Carrier” = produced at least 1 affected puppy OR has at least 1 affected parent
    2. “Not carrier” = has no affected puppies or parents
3. Calculate & evaluate % incidence with various parent combinations:
    1. Affected x Affected (if applicable)
    2. Affected x “Carrier” (if applicable)
    3. "Carrier” x “Carrier”
    4. "Carrier” x “Not carrier”
    5. "Not carrier” x “Not carrier”



Where could this go wrong?

Problem	Consequence	Solution
Poor quality measurements (inconsistent or inaccurate)	Evaluations and therefore decisions will be inaccurate (Garbage in = garbage out)	Ensure consistent, accurate measurements
Only have information on breeders	Can't tell which breeders/families have produced more or less of the issue	Screen as many dogs as possible
Dogs' health is not followed throughout their lifetimes	Don't know which dogs are affected, especially for later-onset conditions	Continue collecting information about dogs' health as they age, e.g., with an annual health survey, regular screenings, etc.
No record made when dog is examined and found to be normal	Can't distinguish dogs confirmed to be healthy from dogs with health unknown	Routinely record "normals"

Considerations if risk is suggested

Replacement Breeder Selection

- How many siblings are affected?
  - Look for 80% or more NORMAL in a litter, as a general rule of thumb.
  - Depends on incidence in colony
- Are parents affected?
- How much do you know about the disease?
- **Be sure you're still saving enough dogs!** (Save best available in the number needed from variety of pedigrees.)

Mate Decisions

- Choose mates that reduce risk while following key principles:
  1. Proposed litter has the lowest inbreeding of choices available
  2. Avoid producing affected dogs where genetic tests are available
  3. Avoid repeat matings
  4. Use studs equally
  5. Stud is good for traits where brood is not so good

What to do next?

- Create (& document) guidelines based on incidence and how many issues being managed. Adjust criteria based on accumulated knowledge of disease.

- Follow incidence:
  - If responding, consider pursuing EBVs.
  - If not responding, re-evaluate guidelines/criteria.

Evaluating Risk in Two Broods

Breeder	Bite Prob	Bite Prob Litter	Bite Prob Produced	% Bite Prob Produced	CLD Affected	CLD Produced	% CLD Produced	Sire CLD Affected	Dam CLD Affected
Brio	No	1	2	0.11	No	0	0	No	No
Capri	No	0	0	0	Yes	1	0.08	No	Yes

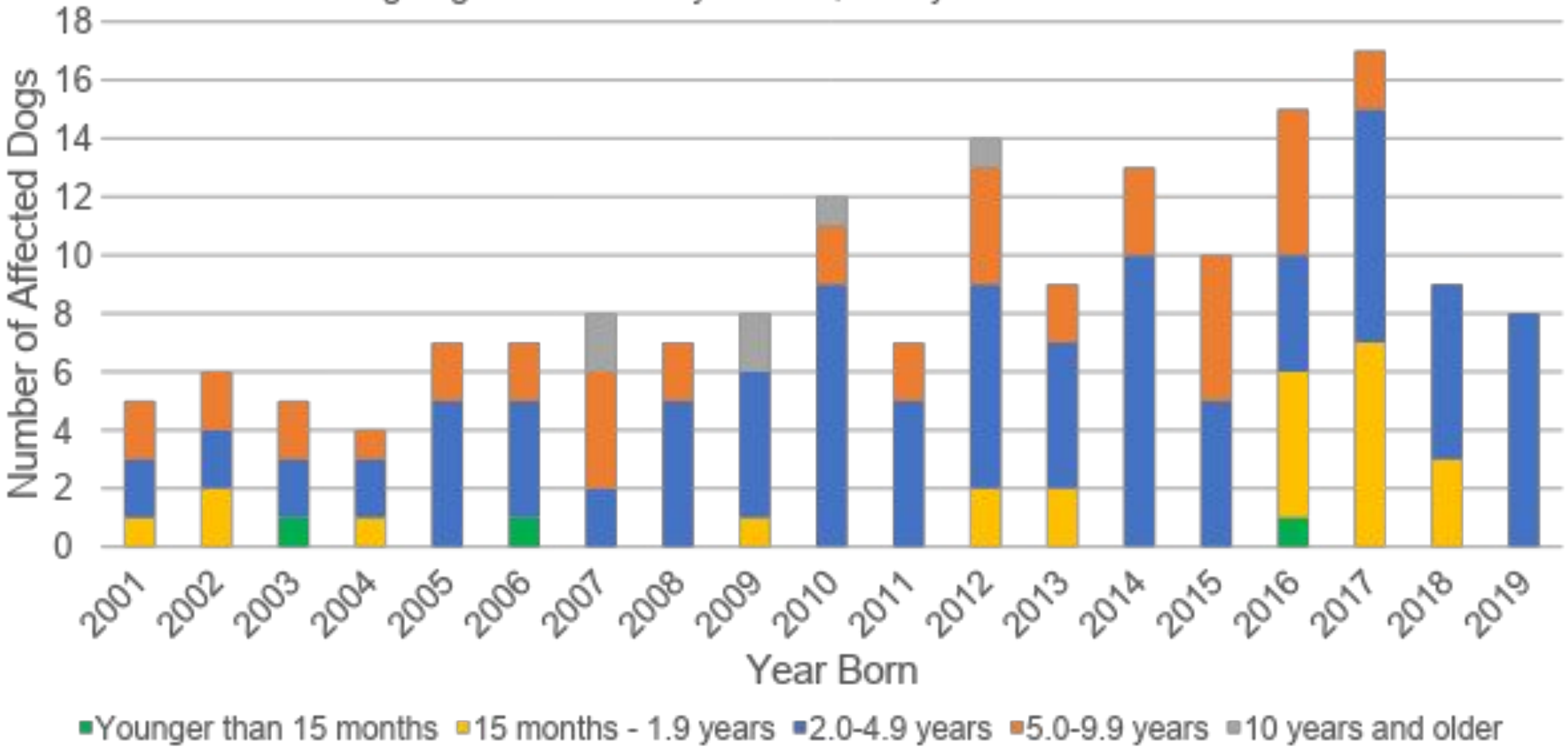
Breeder	Cleft Litter	Cleft Produced	% Cleft Produced	Curled Toes	Curled Toes Litter	Curled Toes Produced	% Curled Toes Produced	Entropion Affected	Entropion Produced	% Entropion Produced
Brio	0	0	0	0	0	1	0.06	0	0	0
Capri	0	0	0	0	0	0	0	0	1	0.03

Breeder	Head Tremor Affect	Head Tremor Litter	Head Tremor Prod	% Head Tremor Prod	Iris Cyst Score	Liver Shunt Sib	Liver Shunt Produced	% Liver Shunt Produced	Skin Produced	% Skin Produced
Brio	0	0	1	0.06	5	0	0	0	0	0
Capri	0	0	0	0	5	0	0	0	2	0.11

Guiding Eyes for the Blind

Labrador CLD Cases By Age Diagnosed: April 2023

Average age of onset 4-5 years old; birth years >2019 excluded



Guidelines Developed: CLD

- Breeders examined regularly by a board-certified ophthalmologist:
  - **Potential breeders:** before deciding whether to keep – only keep normal
  - **Active breeders:** annually
    - Newly affected: Retire as soon as possible – not necessarily immediately
  - **Retired breeders:** every 1 or 2 years until at least age 8

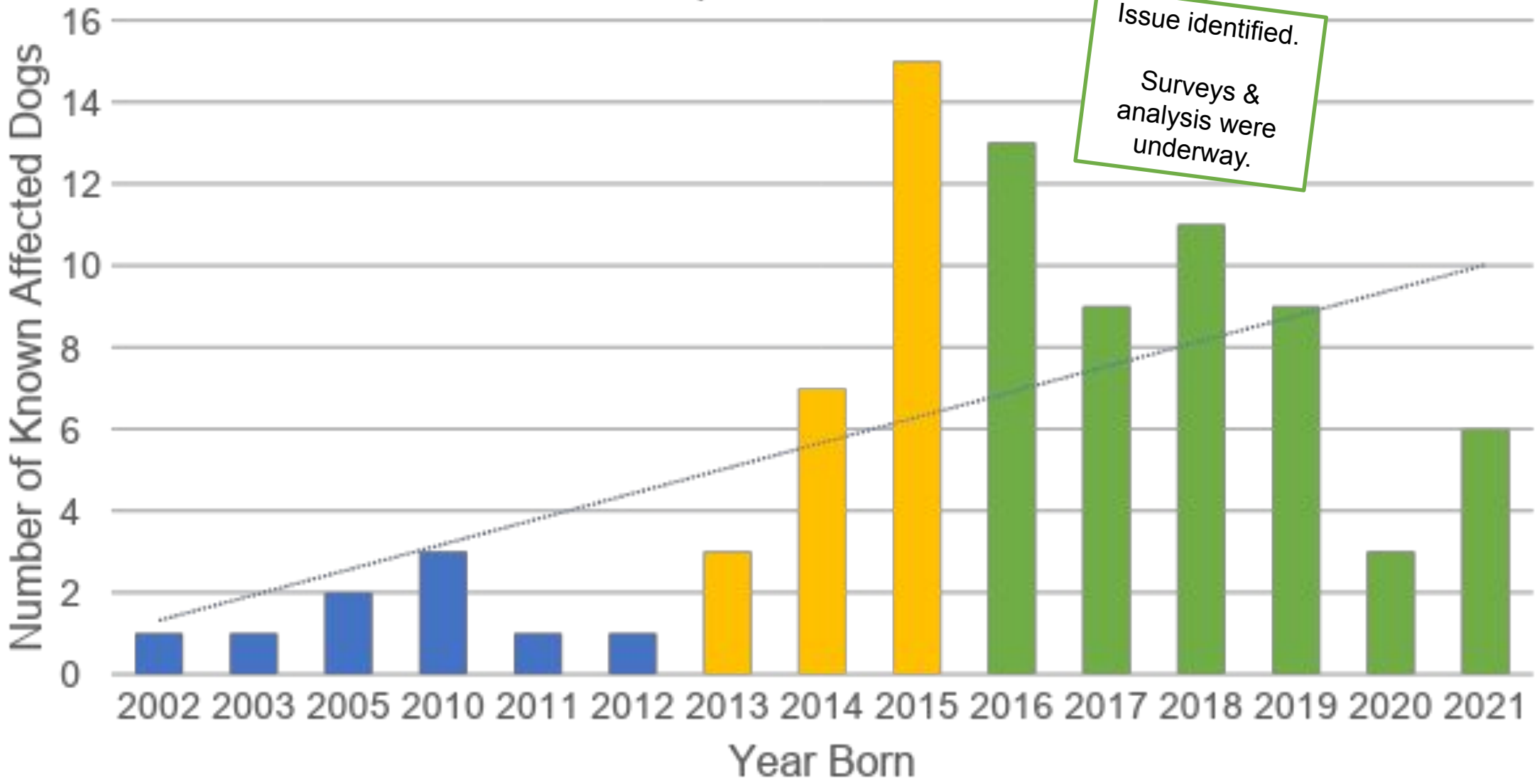
- When possible, avoid mating dogs who both have affected parent(s)

- Collect longitudinal health data on as many dogs as possible.

Guiding Eyes for the Blind

Labrador Head Tremor Cases by Year Born

April 2023



Issue identified.

Surveys & analysis were underway.

Guidelines Developed: Head Tremors

- Avoid breeding littermates of affected dogs. Rare exception: if they are otherwise genetically exceptional, save only 1 brood or limited semen from a male. Do this very sparingly.

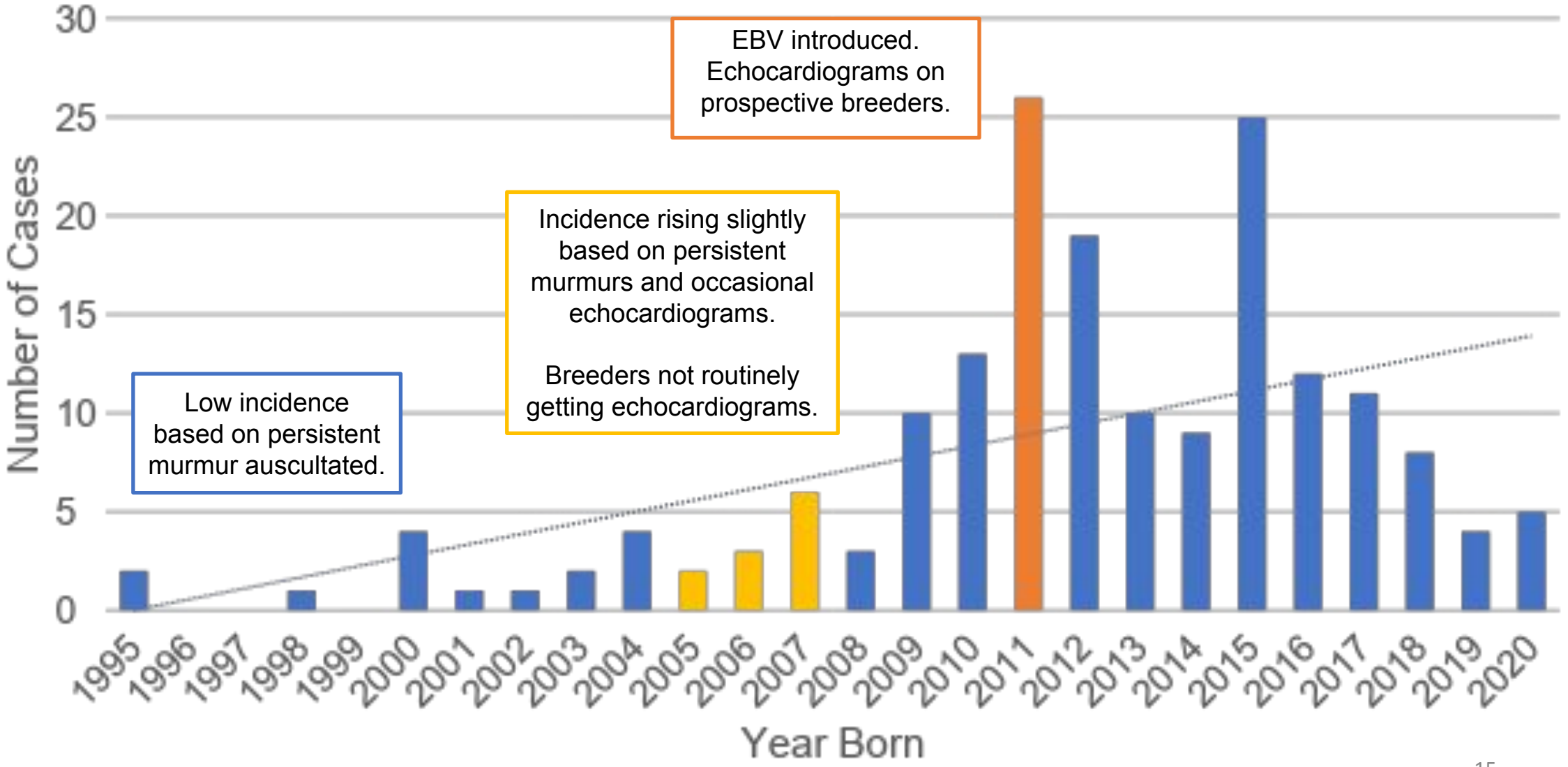
- Avoid mating two dogs with close relatives affected or that have produced many cases.

- Head tremors cases receive follow up contact to confirm details.

Guiding Eyes for the Blind

Labrador TVD Incidence by Year of Birth: April 2023

2021 and 2022 birth years excluded because screening is incomplete.



Guidelines Developed: TVD

- Ensure breeders are normal by echocardiogram – this measure has high heritability.
- Use one (the same) high-quality cardiologist for all screenings for consistency. Different cardiologists may differ in interpreting what is trivial vs significant.
- Have a cardiologist follow up with dogs in field who have a persistent murmur where possible.
- Stop breeding dogs producing TVD at high rates.

Summary

- EBVs are best practice to improve complex traits, but if they are not available, classify dogs according to rough risk assessment (affected, “carrier”, “not a carrier”) and evaluate incidence at different levels of risk in parents. Then develop guidelines for selecting new breeders. Follow up by making mating decisions to lower risk while following genetic principles.

- Monitor trend and revise approach as information evolves.

- Must take best of what’s available in number needed from a variety of pedigrees – there is no perfect dog/family. Risk will be reduced & outcome improved over several generations of selection.

Questions?

Extra Slides

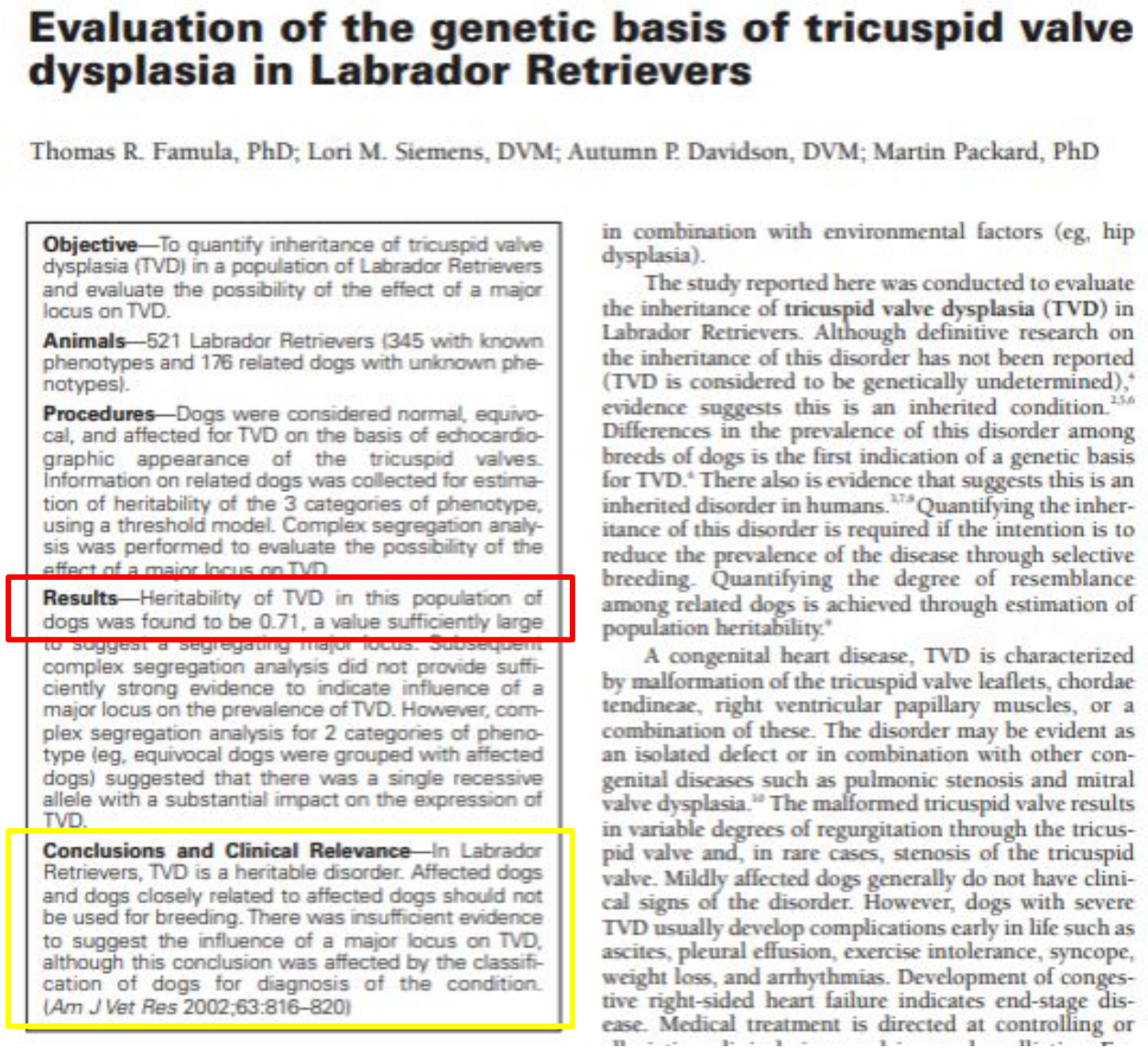
Literature showed TVD to be highly heritable

Famula et al. (2002) reported 71%

GEB EBV (2011) 14%

GEB EBV (2019; different program) 67%

Famula, T. R., Siemens, L. M., Davidson, A. P., & Packard, M. (2002). Evaluation of the genetic basis of tricuspid valve dysplasia in Labrador Retrievers. *American journal of veterinary research*, *63*(6), 816–820. https://doi.org/10.2460/ajvr.2002.63.816



Evaluation of the genetic basis of tricuspid valve dysplasia in Labrador Retrievers

Thomas R. Famula, PhD; Lori M. Siemens, DVM; Autumn P. Davidson, DVM; Martin Packard, PhD

Objective—To quantify inheritance of tricuspid valve dysplasia (TVD) in a population of Labrador Retrievers and evaluate the possibility of the effect of a major locus on TVD.

Animals—521 Labrador Retrievers (345 with known phenotypes and 176 related dogs with unknown phenotypes).

Procedures—Dogs were considered normal, equivocal, and affected for TVD on the basis of echocardiographic appearance of the tricuspid valves. Information on related dogs was collected for estimation of heritability of the 3 categories of phenotype, using a threshold model. Complex segregation analysis was performed to evaluate the possibility of the effect of a major locus on TVD.

Results—Heritability of TVD in this population of dogs was found to be 0.71, a value sufficiently large to suggest a segregating major locus. Subsequent complex segregation analysis did not provide sufficiently strong evidence to indicate influence of a major locus on the prevalence of TVD. However, complex segregation analysis for 2 categories of phenotype (eg, equivocal dogs were grouped with affected dogs) suggested that there was a single recessive allele with a substantial impact on the expression of TVD.

Conclusions and Clinical Relevance—In Labrador Retrievers, TVD is a heritable disorder. Affected dogs and dogs closely related to affected dogs should not be used for breeding. There was insufficient evidence to suggest the influence of a major locus on TVD, although this conclusion was affected by the classification of dogs for diagnosis of the condition. (*Am J Vet Res* 2002;63:816–820)

in combination with environmental factors (eg, hip dysplasia).

The study reported here was conducted to evaluate the inheritance of **tricuspid valve dysplasia (TVD)** in Labrador Retrievers. Although definitive research on the inheritance of this disorder has not been reported (TVD is considered to be genetically undetermined),[4] evidence suggests this is an inherited condition.[2,5,6] Differences in the prevalence of this disorder among breeds of dogs is the first indication of a genetic basis for TVD.[4] There also is evidence that suggests this is an inherited disorder in humans.[3,7,8] Quantifying the inheritance of this disorder is required if the intention is to reduce the prevalence of the disease through selective breeding. Quantifying the degree of resemblance among related dogs is achieved through estimation of population heritability.[4]

A congenital heart disease, TVD is characterized by malformation of the tricuspid valve leaflets, chordae tendineae, right ventricular papillary muscles, or a combination of these. The disorder may be evident as an isolated defect or in combination with other congenital diseases such as pulmonic stenosis and mitral valve dysplasia.[10] The malformed tricuspid valve results in variable degrees of regurgitation through the tricuspid valve and, in rare cases, stenosis of the tricuspid valve. Mildly affected dogs generally do not have clinical signs of the disorder. However, dogs with severe TVD usually develop complications early in life such as ascites, pleural effusion, exercise intolerance, syncope, weight loss, and arrhythmias. Development of congestive right-sided heart failure indicates end-stage disease. Medical treatment is directed at controlling or

2. Choose Mates Where:

1. Proposed litter has the lowest inbreeding of choices available
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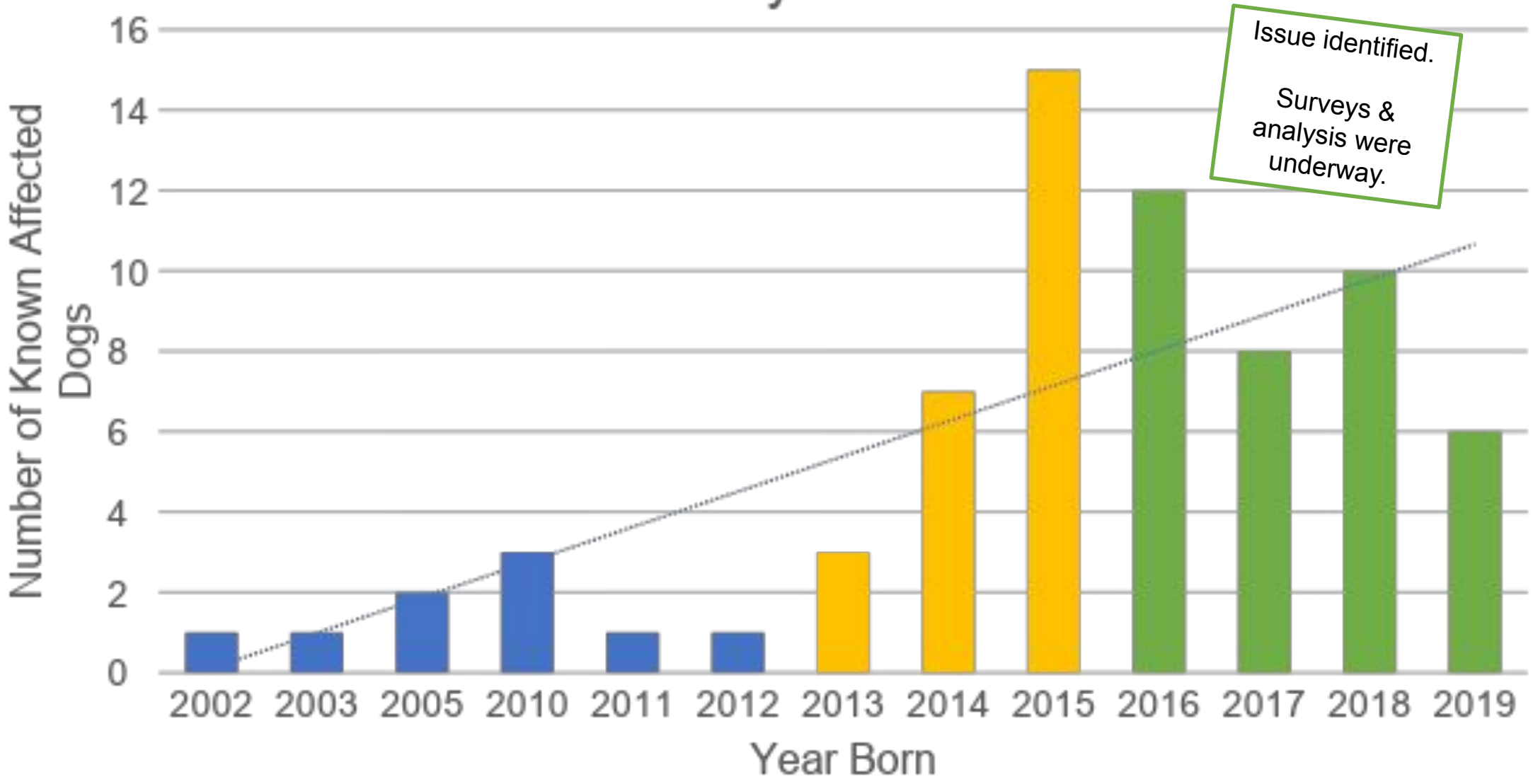
Brood	Mature Pups		Skin	Hip	Elbow	EIC
Addison	15		92.5	82	95.9	C

Stud	# Matings	Litter Inbreeding Coefficient	Skin	Hip	Elbow	EIC
Archer	3	.062	39	79.3	41.8	NP
Carbon	1	.049	76.9	95.6	90.3	NP
Declan	7	.088	49.9	49.4	64.8	NP
Gibson	3	.035	81.8	99.9	95.8	NP
Hero	2	.062	92.7	94.3	79.9	C
Maestro	6	.109	97.6	92.5	94.8	C

Guiding Eyes for the Blind

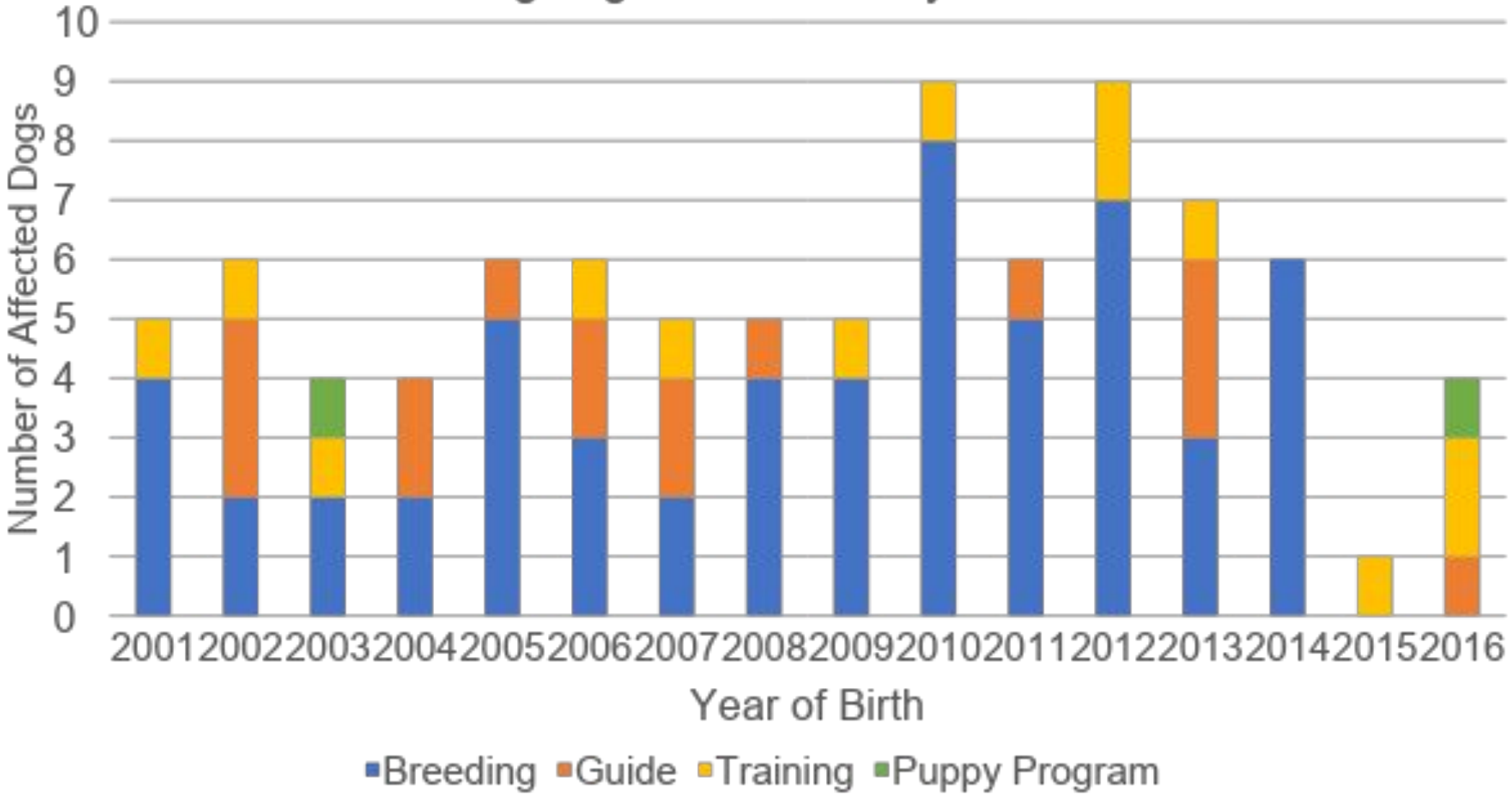
Labrador Head Tremor Cases by Year Born
January 2021



Guiding Eyes for the Blind

Labrador CLD Cases By Program: June 2018

Average age of onset 4-5 years old



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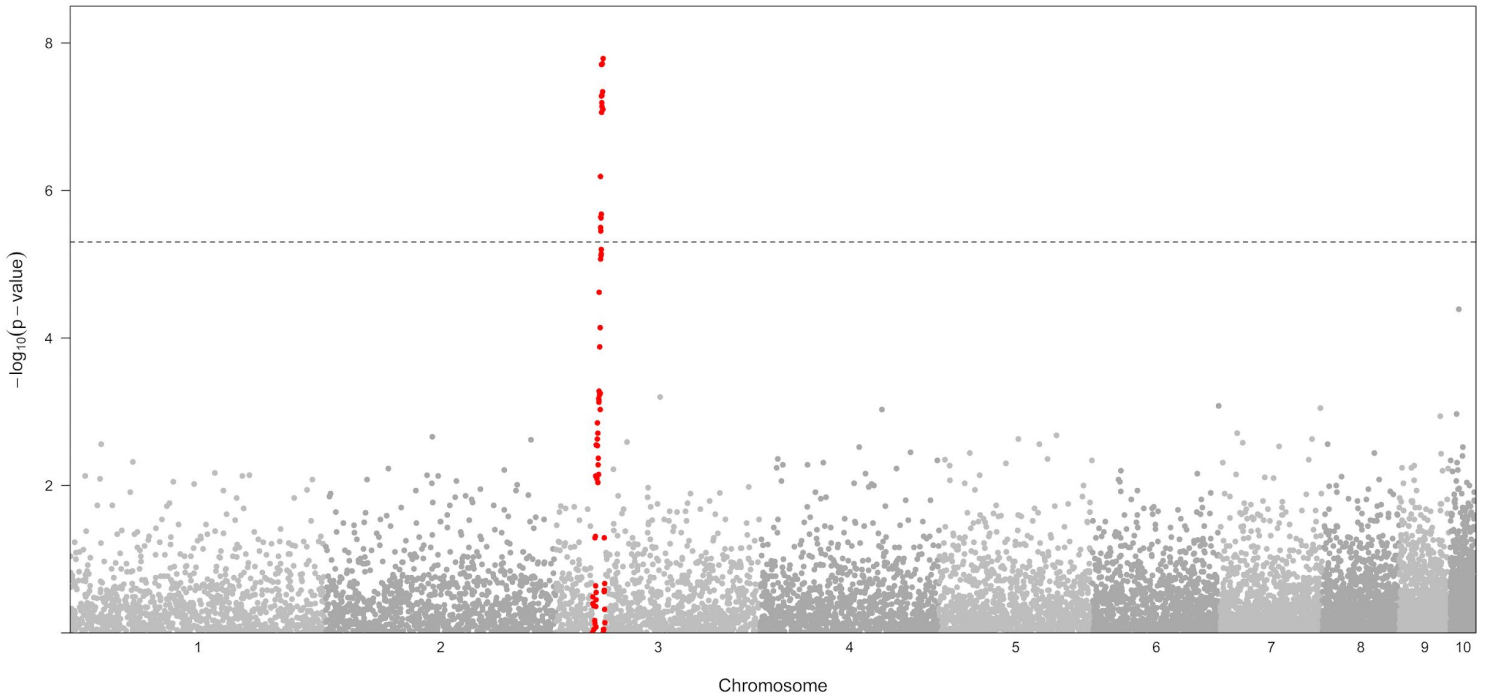
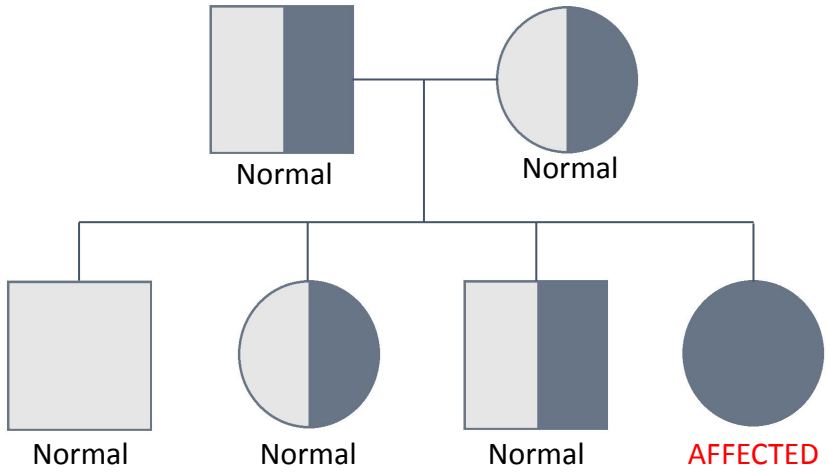
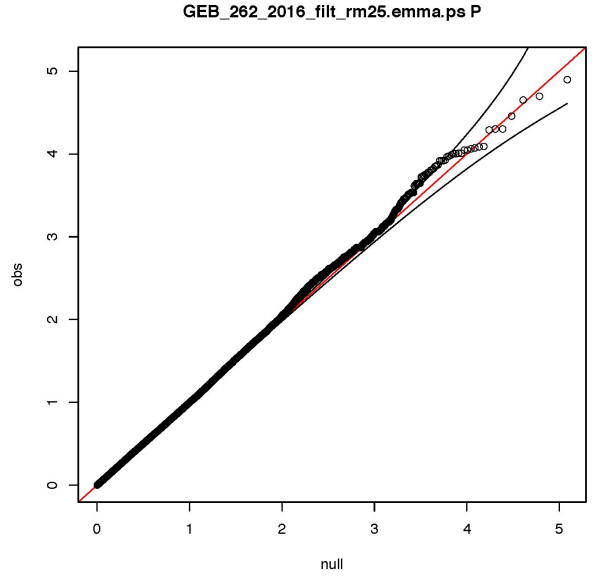


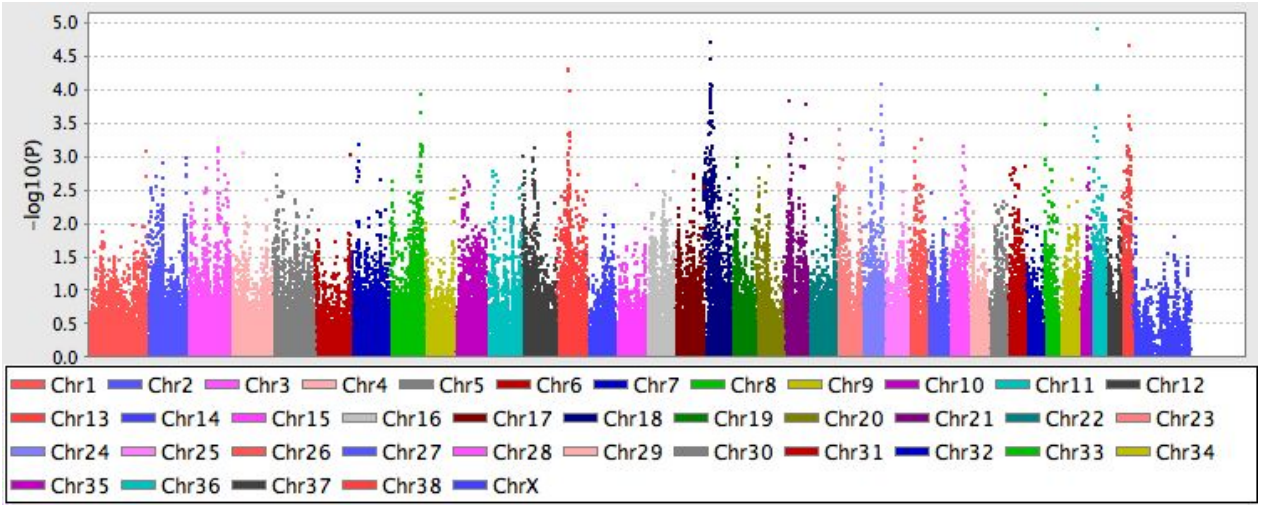
Above data are simulated. Real-life example: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3664012/

	A	a
a	Aa	aa
a	Aa	aa



GEB GWAS analysis based on mast cell tumour cases and controls





•No significant association identified

261 individuals 141 cases 120 controls (recently updated phenotypes) after removing individuals related more than 25% there are 101 cases and 64 controls. Analysis performed using the emmax software. No deviations from the expected p-values in the qq-plot are seen.

- QQ plot to the left shows expected versus observed p-values, a deviation from the expected normal indicates significance. 95% confidence interval is indicated by thin black line.
- Manhattan plot to the right shows dots for each SNP in the analysis. Location of each SNP is on the x-axis and on the y-axis the -10log p-value is noted.

An Autosomal Recessive Trait

- Assume a condition is caused by 1 gene with 2 variants (“A” or “a”):
  - A = normal
  - a = causative

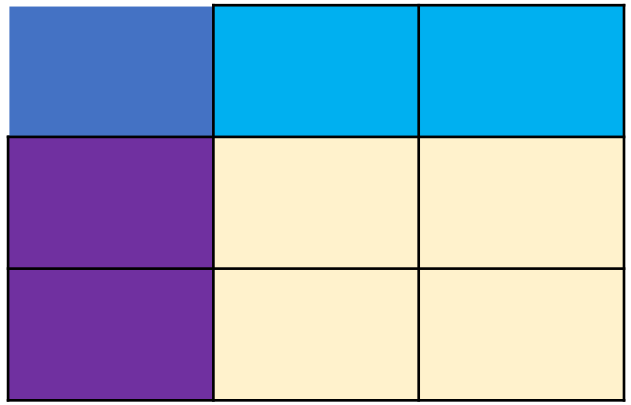
Genotype:	AA	Aa	aa
Phenotype:	Normal	Normal	AFFECTED

Based on the parents’ genotypes, the expected percent of normal vs affected offspring can be easily quantified.

What % incidence should be expected?

Genotype:	AA	Aa	aa
Phenotype:	Normal	Normal	AFFECTED
	Clear	Carrier	Affected

Parent 2 Genotype



Parent 1 Genotype

Possible puppy genotypes
(Each square = 25%)

Example: Carrier x Affected

Genotype:	AA	Aa	aa
Phenotype:	Normal	Normal	AFFECTED
	Clear	Carrier	Affected

	A	a

Parent 1 Genotype

Parent 2 Genotype

Possible puppy genotypes
(Each square = 25%)

Example: Carrier x Affected

Genotype:	AA	Aa	aa
Phenotype:	Normal	Normal	AFFECTED
	Clear	Carrier	Affected

Parent 2 Genotype

	A	a
	A	a
	A	a

Parent 1 Genotype

Possible puppy genotypes
(Each square = 25%)

Example: Carrier x Affected

Genotype:	AA	Aa	aa
Phenotype:	Normal	Normal	AFFECTED
	Clear	Carrier	Affected

	A	a
a	A	a
a	A	a

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	Clear	Carrier	Affected

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Example: Carrier x Affected

Genotype:	AA	Aa	aa
Phenotype:	Normal	Normal	AFFECTED
	Clear	Carrier	Affected

Parent 2 Genotype

	A	a
a	Aa	aa
a	Aa	aa

Parent 1 Genotype

Possible puppy genotypes
(Each square = 25%)
Aa = 25% + 25% = 50% Normal
aa = 25% + 25% = 50% Affected

What % incidence should be expected?

Parent Combination		Expected % Affected Progeny
Affected x Affected	aa x aa	**100%**
Affected x Carrier	aa x Aa	**50%**
Affected x Clear	aa x AA	0%
Carrier x Carrier	Aa x Aa	**25%**
Carrier x Clear	Aa x AA	0%
Clear x Clear	AA x AA	0%

Traits of interest are usually more complicated and therefore rarely follow this exactly.

If parents are grouped into roughly Affected, presumed "Carrier," and presumed "Not a carrier," there may be observed increases in incidence among litters with presumed higher risk parents.