DNA Tests – how to spot a good one and make the most of it

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THE KENNEL CLUB GENETICS CENTRE
AT THE ANIMAL HEALTH TRUST

September 2012

Talk Layout

- What is a DNA test?
- What is the role of the DNA test in dog breeding?
- What requirements do DNA tests need to meet to fulfil their role?
- Examples of DNA tests – the good and the not so good
- Who are the various stakeholders in DNA test development and delivery and what are respective roles?

What is a DNA test?

- A DNA test gives information about the DNA sequence of an individual dog at a position in the genome that is known to influence a particular inherited trait.
- Most DNA tests are for a specific mutation that has been shown to cause an inherited disorder.
- Sometimes the mutation causes a 'cosmetic' trait, such as coat colour.
- Currently, most DNA tests are for mutations that cause 'Mendelian' or genetically simple inherited disorders.
- Increasingly DNA tests will become available for 'genetic risk factors, which increase an individual's risk of developing a disorder but are not the 'whole story'.

Mutations

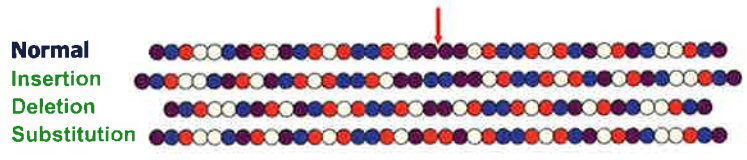
A mutation is a permanent change to the nucleotide sequence of an organism's DNA

Types of mutation:

Insertion – the addition of one or more extra nucleotides into the DNA

Deletion – the loss of one or more extra nucleotides from the DNA

Substitution – the exchange of a single nucleotide

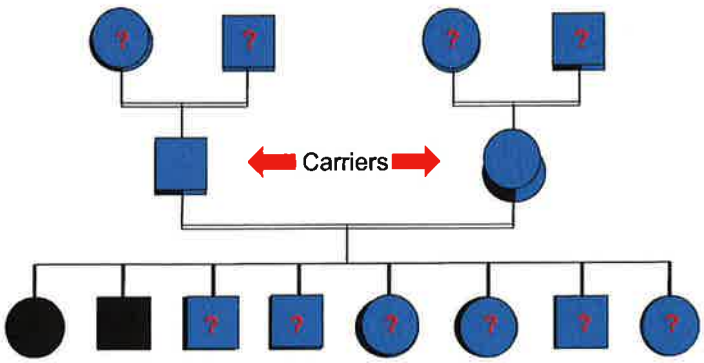


Mutations can be small-scale, involving single, or small numbers of nucleotides, or large, involving hundreds, or thousands of nucleotides.

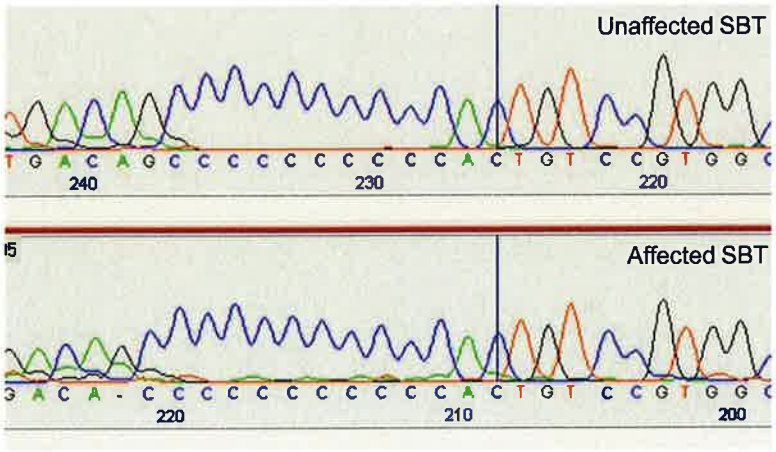
GCACGTACTGAACGT

Recessive Disorders

- Carriers of recessive mutations are clinically free of disease
- Matings between 2 carriers produce affected, carrier & clear offspring in the ratio of 1:2:1

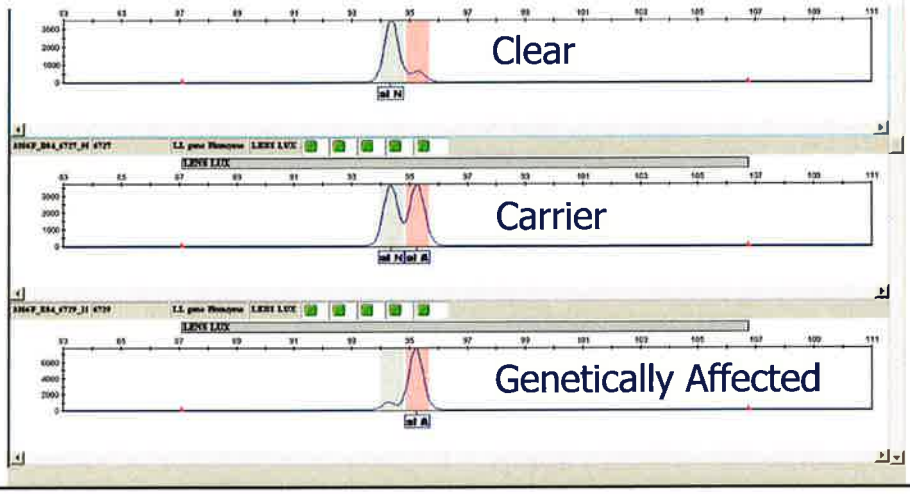


HC Mutation in HSF4



Normal DNA	GACAGCCCCCCCCCCACTGTCC
Mutation	GACAGCCCCCCCCCCCCACTGTCC

DNA Test Results



Simple vs Complex

- ***Mendelian*** or ***simple*** disorders are caused by single mutations.

  Genotype = Phenotype

- Simple disorders can be recessive or dominant, autosomal or sex-linked
- ***Complex*** disorders are caused by multiple mutations or the interaction between genes and the environment.

  Genotype ***predicts*** Phenotype

- Currently, most DNA tests are for mutations that cause simple disorders
- DNA tests based on mutations ***associated*** with complex disorders will probably become increasingly available over coming years.

What is the role of the DNA test?

Individual breeder wants to ....

The Breed needs to ....

.... eliminate the mutation from their line/kennel

.... reduce the frequency of the mutation in the population

.... minimise the risk of producing clinically affected puppies

.... maintain genetic diversity

Individual breeder wants to....

.... minimise the risk of producing clinically affected puppies

Need to know ...

i) ...how common is this mutation, in other words... do I need to test for it?

ii) ... if the disorder is simple or complex, in other words... how well does the genotype predict phenotype?
This is sometimes described as the genotype-associated risk.

iii) ... what is the mode of inheritance of the disorder?

Things to Think About

Before using a DNA test consider…

1. Is information available regarding the prevalence of the disorder, or the frequency of the mutation in the breed? Do I need to test for this mutation?
2. Is information available regarding the disease-associated risk of each genotype?
3. Is information available regarding the mode of inheritance?

Individual breeder wants to ….

The Breed needs to ….

…. eliminate the mutation from their line/kennel

…. reduce the frequency of the mutation in the population

Requires…

…. accurate DNA test that reliably determines genotype, so that's dogs that are free of the mutation can be selected to breed on from.

Things to Think About

Before using a DNA test consider…

1. Is information available regarding the prevalence of the disorder, or the frequency of the mutation in the breed? Do I need to test for this mutation?
2. Is information available regarding the disease-associated risk of each genotype?
3. Is information available regarding the mode of inheritance?
4. Has the DNA testing laboratory got a good reputation for accuracy and for a willingness to help resolve queries? ISO accreditation? Was the testing laboratory involved with the research to identify the mutation?

The Breed needs to….

…. maintain genetic diversity

Requires…

!! not trivial !!

… breeding strategy based on:

i) disease-associated risk of each genotype

ii) mutation frequency within population

iii) (ideally) population structure

Things to Think About

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1. Is information available regarding the prevalence of the disorder, or the frequency of the mutation in the breed? Do I need to test for this mutation?
2. Is information available regarding the disease-associated risk of each genotype?
3. Is information available regarding the mode of inheritance?
4. Has the DNA testing laboratory got a good reputation for accuracy and for a willingness to help resolve queries? ISO accreditation? Was the testing laboratory involved with the research to identify the mutation?
5. What am I going to do with the results? How will the results affect my breeding decisions?

Estimating Mutation Frequency

- Knowing the mutation frequency when a DNA test is made available allows progress to be monitored over the years.
- DNA testing advice can be customised – should a DNA test be required, recommended, etc.
- Breeding advice regarding carriers can be customised.

Estimating Mutation Frequency

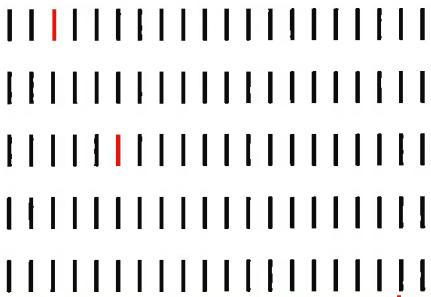
- Neither the **research** or **DNA tested** sample sets available to us are likely to be truly representative of UK population.
- Research samples tend to be biased towards affected dogs and their close relatives.
- Samples collected via DNA testing are not 'random' either:

  Owners might be more likely to have their dogs DNA tested if:

  - they think they might have a problem, or
  - they think their dogs are likely to be clear

- **Accurately** determining frequency of a mutation usually requires collection of random subsets of dogs.

Mutation Frequency



1 in 100 chromosomes carries mutation

p = frequency of **normal allele** = 98% or **0.98**

q = frequency of **mutation** = 2% or **0.02**

Probability of being **affected** = probability of having | AND |

= 0.02 **AND** 0.02

= 0.02 x 0.02

= 0.0004 = **4 in 10,000**

Probability of being **clear** = probability of having | AND |

= 0.98 **AND** 0.98

= 0.98 x 0.98 = 0.9604

= ~ **9,604 in 10,000**

Probability of being a Carrier

Carrier = | AND | OR | AND |

= (0.02 x 0.98) or (0.98 x 0.02)

= (0.02 x 0.98) + (0.98 x 0.02)

= 0.0392

= 3.92%

= ~ 392 out of every 10,000

$P^2 + 2pq = q^2 = 1$ = Hardy-Weinburg equilibrium

0.9604 + 0.0392 + 0.0004 = 1

Why breed with carriers?

- Breeding with carriers is recommended if mutation frequency is > 0.01
- **Remember** - the disease mutation for which there is a DNA test is not the only mutation a carrier has.
- If carriers are not bred from and clear dogs are used extensively then there is a real risk that other mutations carried by those 'clear' dogs will increase in frequency in the breed and new inherited disease(s) could emerge.

Mating outcomes for recessive mutations

Combination of Dogs	Outcome	Possibility of clinically affected offspring?
Clear X Clear	All puppies will be clear	No
Clear X Carrier	~ 50% of puppies will be clear ~ 50% of puppies will be carriers	No
Clear x Affected	All puppies will be carriers	No
Carrier x Carrier	~ 25% of puppies will be clear ~ 25% of puppies will be affected ~ 50% of puppies will be carriers	Yes
Carrier x Affected	~ 50% of puppies will be affected ~ 50% of puppies will be carriers	Yes
Affected x Affected	All puppies will be affected	Yes

What to look for in a DNA test

- Have details of the disease-associated mutation been subjected to peer-review? Is the DNA test based on '*good science*'?
- Has the mutation been shown to be disease-associated in **your** breed?
- Is the mode of inheritance discussed?
- Dominant with incomplete penetrance…. is information given the degree of penetrance?
- Is the frequency of the mutation discussed?

- OptiGen DNA test for ***prcd*-PRA**
- Info' given about:
  - Disease ✓
  - Inheritance ✓
  - Breeding strategies ✓

http://www.optigen.com/opt9_test_prcd_pra.html

PRA Disease

The genetic disorder, *prcd*-PRA , causes cells in the retina at the back of the eye to degenerate and die, even though **the cells seem to develop normally early** in life. The "rod" cells operate in low light levels and are the first to lose normal function. Night blindness results. Then the "cone" cells gradually lose their normal function in full light situations. Most affected dogs will eventually be blind. Typically, the clinical disease is recognized first in early adolescence or early adulthood. Since age at onset of disease varies among breeds, you should read specific information for your dog. Diagnosis of retinal disease can be difficult. Conditions that seem to be *prcd*-PRA might instead be another disease and might not be inherited. OptiGen's genetic test assists in making the diagnosis. It's important to remember that not all retinal disease is PRA and not all PRA is the *prcd* form of PRA. Annual eye exams by a veterinary ophthalmologist will build a history of eye health that will help to diagnose disease.

Unfortunately, at this time there is no treatment or cure for PRA. If your dog is affected, you may find it helpful to read about other owners' experiences living with blind dogs. (suggested links www.eyevet.org and www.blinddogs.com)

Inheritance

Prcd-PRA is inherited as a recessive trait. This means a disease gene must be inherited from each parent in order to cause disease in an offspring. Parents were either "carrier" or affected. A carrier has one disease gene and one normal gene, and is termed "heterozygous" for the disease. A normal dog has no disease gene and is termed "homozygous normal" – both copies of the gene are the same. And a dog with two disease genes is termed "homozygous affected" – both copies of the gene are abnormal

It's been proven that all breeds being tested for *prcd*-PRA have the same disease caused by the same mutated gene. This is so, even though the disease might develop at different ages or with differing severity from one breed to another.

Although *prcd*-PRA is inherited, it can be avoided in future generations by testing dogs before breeding. Identification of dogs that do not carry disease genes is the key. These "clear" dogs can be bred to any mate - even to a *prcd*-affected dog which may be a desirable breeding prospect for other reasons. The chance of producing affected pups from such breedings depends on the certainty of test results. Again, you'll find the specific information on certainty of test results for your dog by linking to breed specific information.

The Genetic Test

The OptiGen prcd test is done on a small sample of blood from the dog. The test analyzes the specific DNA mutation causing *prcd*-PRA. The OptiGen test detects the mutant, abnormal gene copy and the normal gene copy. The result of the test is a genotype and allows separation of dogs into three groups: Normal/Clear (homozygous normal), Carrier (heterozygous) and Affected (homozygous mutant).

Possible results using the OptiGen *prcd* test

Genotype	Risk Group	Significance For Breeding	Risk of *prcd* Disease
Homozygous Normal	Normal/Clear	Can be bred to any dog, extremely low risk of producing affecteds	Extremely low
Heterozygous	Carrier	Should be bred only to Normal/ Clear to remove risk of producing affecteds	Extremely low
Homozygous Mutant	Affected	Should be bred only to Normal/ Clear to remove risk of producing affecteds	Very high

Breeding Strategies

Benefits & Limits to All Genetic Testing

The OptiGen *prcd*-PRA Test

The OptiGen *prcd*-PRA test is a DNA-based test that helps you avoid one form of Progressive Retinal Atrophy (PRA). PRA refers to a group of diseases that cause the retina of the eye to degenerate slowly over time. The result is declining vision and eventual blindness. "*prcd*" stands for "progressive rod-cone degeneration" which is the type of PRA known in several breeds. AFTER reading the information on this page, you can link to information specifically about the breed in which you are interested.

Genetic Registries – genetic registries have been established for several breeds. For these breeds results are shared with OFA, CERF or with a breed designated registry. We have noted below with an asterisk which breeds are included. This policy applies only to those registries that are in effect at the time the test is requested.

Breed Links
Breeding Strategies

Breed specific information:

- Cocker Spaniel (American)
- American Eskimo Dog
- Australian Cattle Dog
- Australian Shepherd
- Australian Shepherd, Miniature & Toy
- Australian Stumpy Tail Cattle Dog
- Chesapeake Bay Retriever - *Normals Only for US owned dogs
- Chinese Crested
- Cockapoo
- Dwarf Poodle
- English Cocker Spaniel
- Entlebucher Mountain Dog
- Finnish Lapphund
- Giant Schnauzer
- Golden Retriever
- Golden Doodle
- Karelian Bear Dog
- Kuvasz
- Lab/Golden Cross
- Labradoodle
- Labradoodle, Australian
- Labrador Retriever
- Lapponian Herder
- Markiesje
- Miniature & Toy Poodle
- Miniature American Shepherd
- Moyen Poodle
- Norwegian Elkhound
- Nova Scotia Duck Tolling Retriever - *All US and Canada
- Portuguese Water Dog - *All Results for US owned or PWDCA members
- Silky Terrier
- Spanish Water Dog - *OFA (fee)
- Swedish Lapphund
- Yorkshire Terrier

PRA Disease

The genetic disorder, *prcd*-PRA , causes cells in the retina at the back of the eye to degenerate and die, even though the cells seem to develop normally **early** in life. The "rod" cells operate in low light levels and are the first to lose normal function. Night blindness results. Then the "cone" cells gradually lose their normal function in full light situations. Most affected dogs will eventually be blind. Typically, the clinical disease is recognized first in early adolescence or early adulthood. Since age at onset of disease varies among breeds, you should read specific information for your dog. Diagnosis of retinal disease can be difficult. Conditions that seem to be *prcd*-PRA might instead be another disease and might not be inherited. OptiGen's genetic test assists in making the diagnosis. It's important to remember that not all retinal disease is PRA and not all PRA is the *prcd* form of PRA. Annual eye exams

OPTIGEN for the genetic advantage

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prcd-PRA Test

For: Nova Scotia Duck Tolling Retrievers

Genetic Registries – Please read below about genetic registries established for Tollers. Additional fees may apply.

prcd-PRA - Test General Information
Breeding Strategies
Breed Limits

1. **Is there more than one type of PRA in Tollers?**
Based on experience to date, there is only one form of PRA in Tollers, the form called progressive rod cone degeneration (*prcd*). Thus, the DNA mutation test is expected to identify all cases of Toller PRA. Several other breeds have this same type of PRA, however the typical characteristics of the disease can be somewhat different from breed to breed, for example, age of onset, severity, and rate of progression to blindness. Tollers show much variation in each of these characteristics.

2. **What is the usual age at diagnosis?**
Tollers have been diagnosed with PRA over a very wide age range – as young as 3 years and as old as 8 years. The typical age of diagnosis is 4 to 6 years. Some dogs are quite old before the disease is seen. Other dogs might never show signs of PRA even though they are genetically affected. As more dogs are examined, it's likely that even younger and older dogs will be discovered showing first signs of PRA.

3. **Are there any proven Toller cases of false positive in this test similar to other breeds?**
So far there is no known case of a false positive in Tollers. There is no evidence so far that a Carrier dog might actually be Normal/Clear, nor that an Affected dog might actually be Carrier or even Normal/Clear. This situation is very different than for the initial *prcd*-PRA test in most other breeds where the rate of false positives was substantial. In Tollers, both "expressivity" and "penetrance" of PRA play an important role in understanding PRA genetic status

4. **What is variable expressivity?**
Some diseases are very predictable in terms of age of onset and severity of symptoms. Such a disease is typically "expressed" in the same way in each affected individual. But Toller PRA doesn't fit this description. It can have different ages of onset, different degrees of severity, and/or different rates of progression within the same line, the same pedigree, or even the same litter. One confusing result of reduced or variable expressivity is that a dog can test Affected, yet show no clinical signs of abnormal vision until much later, or show only mild and slowly progressing clinical signs of the disease. This dog must not be confused with a case of false positive.

5. **What is penetrance?**
The extreme case of reduced expressivity is incomplete penetrance. An inherited disease has incomplete penetrance in

http://www.aht.org.uk/cms-display/genetics_success.html — Canine success stories | Ani... — aht.org.uk

Episodic Falling and Dry Eye and Curly Coat Syndrome

Episodic falling (EF) in the Cavalier King Charles Spaniel (CKCS) is a serious, debilitating, inherited condition that is distressing for both affected dogs and their owners. EF is an inherited 'exercise-induced hyperlonicity disorder' meaning that there is increased muscle tone and the muscles are unable to relax. Episodes usually occur in response to excitement, exercise, or frustration, except in severe cases in which symptoms may be chronic or happen with no apparent cause. Clinical signs usually appear before one year of age and both male and female dogs are affected.

Dry eye and curly coat syndrome, known clinically as congenital keratoconjunctivitis sicca and ichthyosiform dermatosis, affects a dog's eyes and skin. Affected dogs produce no tears making their eyes incredibly sore. Their skin becomes very flaky and dry, particularly around the foot, and this can make standing and walking difficult and painful. This syndrome appears to be a problem unique to CKCS and many dogs diagnosed with the condition are put to sleep.

With funding from the WALTHAM Foundation (Pedigree Masterfoods), the Kennel Club Charitable Trust and the Tasmae Charitable Trust we have identified mutations associated with both of these conditions, and DNA tests are now available. Further research has now been completed to calculate the frequency of the two mutations in the CKCS population in the UK. To download a copy of the report that describes the study, our findings and our recommendations please Click here. For more information please contact Lou Hayward

Reference

Parallel Mapping and Simultaneous Sequencing Reveals Deletions in BCAN and FAM83H Associated with Discrete Inherited Disorders in a Domestic Dog Breed

Forman OP, Penderis J, Hartley C, Hayward LJ, Ricketts SL, Mellersh CS (2012)

PLoS Genetics 8(1): e1002462. doi:10.1371/journal.pgen.1002462

Open access available at: http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1002462

- AHT DNA test for ***EF and DE/CC:***
- Info' given about:
  - Disease ✓
  - Publication ✓
  - Mutation frequency and breeding recommendations ✓

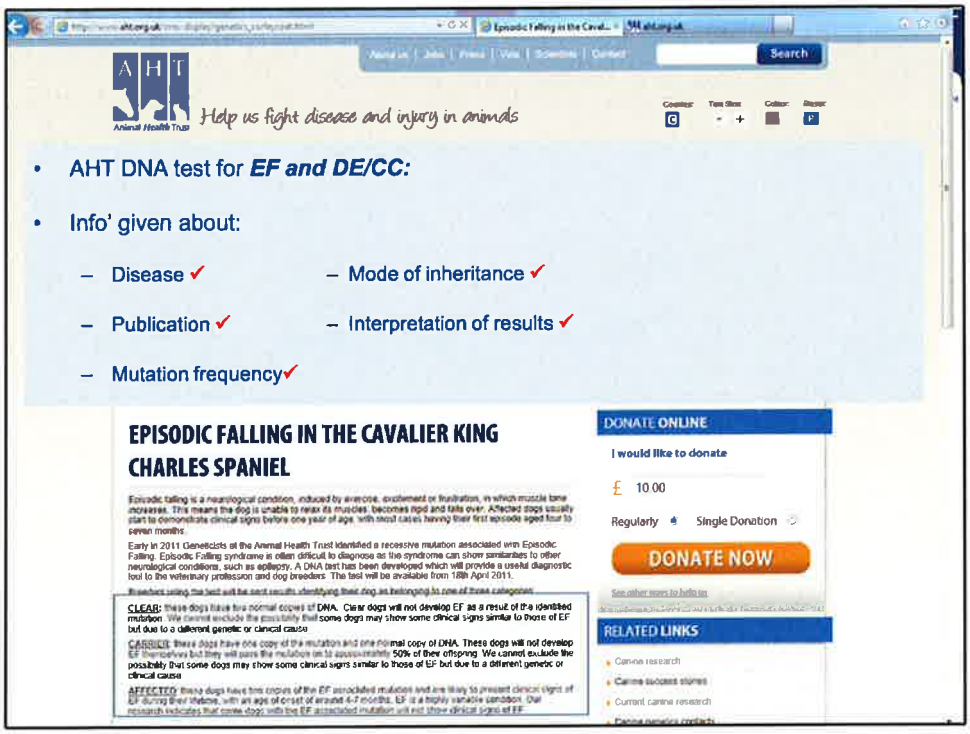
AHT
Animal *Health* Trust

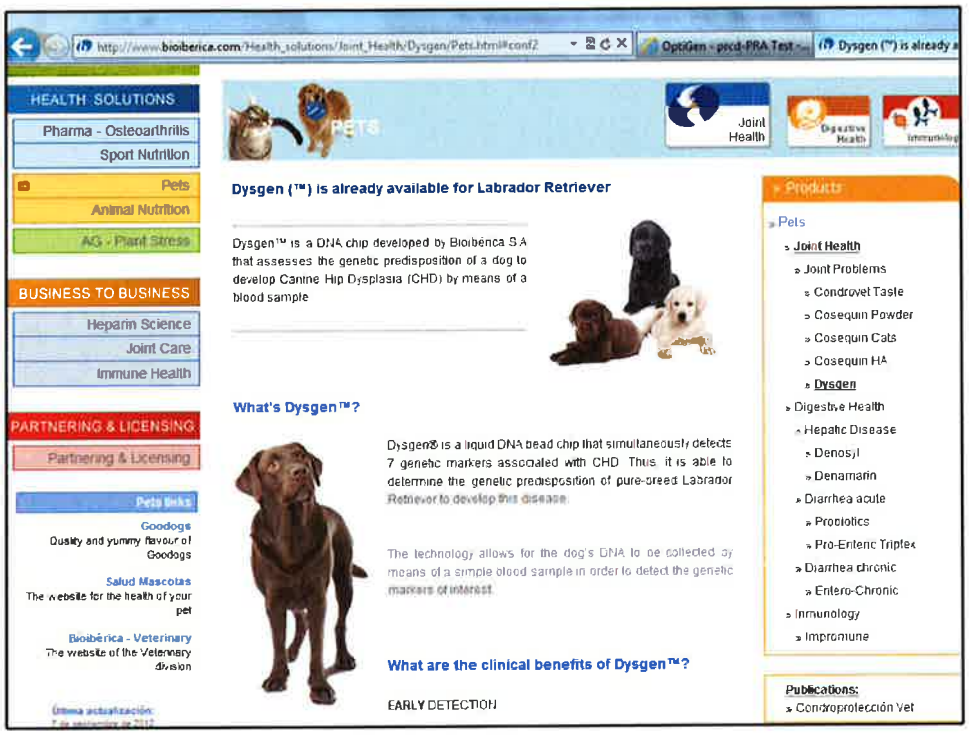
Animal Health Trust

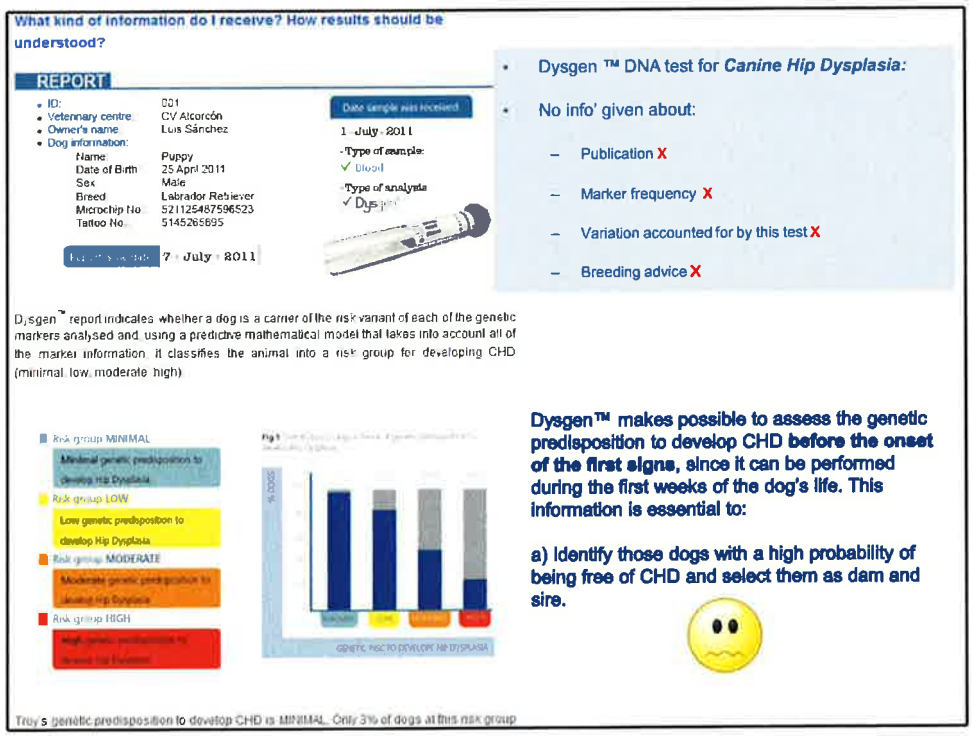
THE KENNEL CLUB GENETICS CENTRE
AT THE ANIMAL HEALTH TRUST

Frequency of two disease-associated mutations in Cavalier King Charles Spaniels

June 2012




HEALTH SOLUTIONS
Pharma - Osteoarthritis
Sport Nutrition
Pets
Animal Nutrition
AG - Plant Stress
BUSINESS TO BUSINESS
Heparin Science
Joint Care
Immune Health
PARTNERING & LICENSING
Partnering & Licensing
Pets links
Goodogs
Quality and yummy flavour of Goodogs
Salud Mascotas
The website for the health of your pet
Bioibérica - Veterinary
The website of the Veterinary division
PETS
Joint Health
Dysgen (™) is already available for Labrador Retriever
Dysgen™ is a DNA chip developed by Bioibérica S.A that assesses the genetic predisposition of a dog to develop Canine Hip Dysplasia (CHD) by means of a blood sample
What's Dysgen™?
Dysgen® is a liquid DNA bead chip that simultaneously detects 7 genetic markers associated with CHD. Thus, it is able to determine the genetic predisposition of pure-breed Labrador Retriever to develop this disease.
The technology allows for the dog's DNA to be collected by means of a simple blood sample in order to detect the genetic markers of interest.
What are the clinical benefits of Dysgen™?
EARLY DETECTION
Products
Pets
Joint Health
Joint Problems
Condrovet Taste
Cosequin Powder
Cosequin Cats
Cosequin HA
Dysgen
Digestive Health
Hepatic Disease
Denosyl
Denamarin
Diarrhea acute
Probiotics
Pro-Enteric Triptex
Diarrhea chronic
Entero-Chronic
Inmunology
Impromune
Publications:
Condroprotección Vet


What kind of information do I receive? How results should be understood?
REPORT
ID: 001
Veterinary centre: CV Alcorcón
Owner's name: Luis Sánchez
Dog information:
Name: Puppy
Date of Birth: 25 April 2011
Sex: Male
Breed: Labrador Retriever
Microchip No: 521125487596523
Tattoo No: 5145265695
7 July 2011
Date sample was received
1 July 2011
Type of sample:
Blood
Type of analysis
Dysgen
Dysgen™ report indicates whether a dog is a carrier of the risk variant of each of the genetic markers analysed and, using a predictive mathematical model that takes into account all of the marker information, it classifies the animal into a risk group for developing CHD (minimal, low, moderate, high).
Risk group MINIMAL
Minimal genetic predisposition to develop Hip Dysplasia
Risk group LOW
Low genetic predisposition to develop Hip Dysplasia
Risk group MODERATE
Moderate genetic predisposition to develop Hip Dysplasia
Risk group HIGH
High genetic predisposition to develop Hip Dysplasia
GENETIC RISK TO DEVELOPE HIP DYSPLASIA
Troy's genetic predisposition to develop CHD is MINIMAL. Only 3% of dogs at this risk group
Dysgen ™ DNA test for Canine Hip Dysplasia:
No info' given about:
Publication X
Marker frequency X
Variation accounted for by this test X
Breeding advice X
Dysgen™ makes possible to assess the genetic predisposition to develop CHD before the onset of the first signs, since it can be performed during the first weeks of the dog's life. This information is essential to:
a) Identify those dogs with a high probability of being free of CHD and select them as dam and sire.

Note of caution

- The number of genetic variants associated with inherited canine disorders will increase dramatically in coming years.
- Many will be ***risk factors*** as opposed to fully penetrant causal mutations associated with simple conditions.
- If these are common within breeds they need to be eliminated ***slowly*** and ***carefully*** to avoid reducing genetic diversity.
- If DNA tests are based on variants with minor roles in disease development there is a real risk that collateral damage to diversity by inappropriate elimination strategies will outweigh benefit gained by reduction in disease prevalence.
- Many gene–disease associations are intriguing & worthy of publication but **not all** are appropriate for a DNA test.

Who should do what?

Stakeholders	Role & responsibility
• Researcher →	•mutation identification •disease-associated risk of each genotype
• Testing laboratory →	•accurate DNA testing service
• Individual breeder →	•use the DNA test •make sensible breeding decisions
• Breed Club & Kennel Club →	•Disseminate information about test •Facilitate researcher with follow-up studies, e.g. recruit random dogs to estimate mutation frequency •Collate DNA test results


Thank you for listening