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# DNA DIAGNOSTIC TESTS FOR INHERITED DISEASES IN PETS

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## Introduction

Knowledge on structure and function of the pet genome is daily increasing. Cat and dog genome have already been sequenced and resources necessary are developed to map and clone genes in an effort to utilize pets as a model system for genetics and cancer research, for infectious disease (including AIDS) and species diversity evolution (1, 2).

In the last few years this huge amount of information hesitated in applicative spin offs. At present, approximately 64 and 24 phenes have been characterized at molecular level in dogs and cats respectively, including popular coat traits, (3, 4, 5) and many inherited diseases (6). For simple genetic disorders direct detection of causative mutations is the most specific method of carrier detection. Molecular analysis on DNA also allow the genetic profiling and parentage control, extremely useful for animal selection and for customer protection. The DNA test results are independent of the age of the animals; thus, the tests can be performed at any time. DNA is very stable, only very small quantities are needed for testing and can be easily stored for further genetic analysis.

## Sampling and conservation

Any DNA analysis starts with nuclear cells sampling. In pets the easiest and more common sources of nuclear cells are peripheral blood draw and buccal swab.

Blood draw is the most common method in dog and consists in a small blood sample (0.5 mls) into an EDTA tube that can be daily mailed without refrigeration in temperate climates. Alternatively in dog it is possible, after buccal puncture, to spot blood drops onto a Vet kard™ (FTA technology; 7), to leave at room temperature until dried and mail into a paper envelope. The quality and quantity of the DNA is poorer in this case. Blood can be conserved at +4°C for 2-4 days or frozen at -20°C for years before sending to the lab.

The buccal swab, also named cheek swab, sample is obtained by twirling 4 swabs inside the cheeks (about 20 to 30 back and forth motions) from the same animal to collect loose buccal cells (not saliva). Hands have to be washed between cat samplings if multiple cats are being sampled. The swabs can be cyto brushes or cotton sticks. The swabs have to be left at room temperature until dried and mailed to the Lab into a paper envelope (for details on buccal samplings: 8). The swabs can be cut and stored at -20°C in a plastic tube, but no data are available on the conservation times. Buccal cells are not a good source of DNA in dogs, while they work well in cats and they are indicated for kittens.

Generally blood is always the best source for the laboratory as provides clean plenty of DNA. Moreover this type of sampling is usually performed in a veterinary clinic and is coupled with a visit of the animal and an identity control, which is important both for sanitary and market reasons.



## Subject identification

In any genetic analysis is of great importance the subject identification. DNA-based genetic testing is used for most domesticated animals to confirm identity and parentage a DNA profile is often included with genetic tests for diseases and traits to ensure that the results pertain to a specific subject. Under the sanitary and research profile the identification of the subject allows the veterinarian to follow it along his life, to monitor the evolution of the disease and to gain important information which can be used for the animal treatment, feeding and breeding. But, more important, a correct identification of the subjects avoids commercial fraud and sets the basis for a correct study and selection of the animals.

Subject identification is feasible in pet both by microchipping and by DNA profiling. Currently DNA profiling, and parentage testing, are performed on standard DNA samples (see above), are based on genetic marker (microsatellite) panels standardized across breeds, worldwide by international comparisons which allow sharing information, combining datasets and assisting with population management, both in dog and cat. These tests are particularly important for purebreds, especially when individuals move between registries and countries. The scientific community provides oversight of industry standards pertaining to DNA profiling and identification panels (9, 10).

## DNA diagnostic tests for inherited diseases

On the same DNA samples used for profiling or parentage analysis is it today possible to identify several causative mutations of inherited diseases. DNA tests can be performed theoretically in newborns, in practice at 2-3 month. Breeders are interested in early typing for choosing the puppets or kittens and for establishing the value of the animals to sell. Moreover customers are beginning to require genetic test certificate together with pedigree when they buy a pet.

So far by DNA genetic tests is possible to spot only disease caused by mutation in one or very few genes. These diseases are defined single trait or Mendelian inherited diseases. The causative mutation occurs in a gene localized in a not sexual chromosome (autosome) or in a sexual chromosome. The effect of the mutant allele can be dominant, affecting the phenotype in single dose (eterozigosity) or it can be recessive, being necessary two mutant alleles of the gene in the same individual (homozygosity) to determine the disease. Many disease pronenesses are caused by multiple gene interaction and environmental factors, such as Hip dysplasia in dogs, however for these defects molecular DNA tests are not available yet.

Inbreeding in purebreds lead to emerging of inherited defects and mutant allele are transmitted by descendent in the breed. It follows that the same disease can occur in different breeds caused by a different mutation in the same gene (such as in Hypertrophic Cardio Myopathy – HCM - in Main Coon and Ragdoll) or in different genes in the same pathway. On reverse, it is possible that the same gene mutation is causative of a defect in different breeds with common ancestors (such as Polycystic Kidney Disease Polycystic Kidney – PKD - in Persian and Exotic).

### *Dog*

More than 30 DNA test are today available for inherited diseases in dogs. Many laboratories provide these tests worldwide (such as 11, 12, 13, 14, 15, 16).



In Table 1 are listed the most common DNA tests commercially available, several are patented and are available only at the Laboratory that holds the exclusive license. Other defects can be tested such as Haemophilia B, Fucosidosis or Copper toxicosis.

Table 1

Acronym	Defect	Breed
CD	Cone degeneration	German Shorthaired Pointers
CEA/CH	Choroidal hypoplasia	Border Collies; Boykin Spaniels; Collie; Lancashire Heelers; Nova Scotia Duck Tolling Retrievers; Rough Collies; Samoyeds; Shetland Sheepdogs; Smooth Collies; Whippets; Longhaired;
CCN	Canine Cyclic Neutropenia (Gray Ccollie Syndrome)	Border Collie, collie
CLAD	Canine Leukocyte Adhesion Deficiency	Irish Setters and Irish Red & White Setters
CMR	Canine Multi-focal Retinopathy	Bulldogs; Coton de Tulear; Dogue de Bordeaux; Great Pyrenees; Mastiffs (Old English)
CSNB	Congenital Stationary Night Blindness	Briards
CYST	Cystinuria	Newfoundlands; Landseer;
FN	Familial nephropathy	English Cocker Spaniels
GCL	Globoid Cell Leukodystrophy	Cairn Terrier , West Highland White Terrier
GM1	GM1-Gangliosidosis	Huskies
JEB	Junctional Epidermolysis Bullosa	German Pointer
L-2-HGA	L- 2 - hydroxyglutaric aciduria	Staffordshire Bull Terrier
MC	Myotonia Congenita	Miniature Schnauzer
MD	Muscular Dystrophy	Golden Retriever
MH	Malignant Hyperthermia	All breeds
MPS	Mucopolysaccharidosis Type VII	German Shepherd
NARC	Narcolepsy	Dachshunds; Doberman Pinschers; Labrador Retrievers
NCL	Neuronal Ceroid Lipofuscinosis	Border Collies
PFK	Phosphofructokinase Deficiency	American and English Cocker Spaniels; Labradoodles Australian;
PK	Pyruvate kinase deficiency	Basenjis; West Highland Terriers
dominant PRA	Progressive Retinal Atrophy	Bulldogs; Mastiffs (Old English);
prcd-PRA		American Cocker Spaniels; American Eskimo Dogs; Australian Cattle Dogs; Australian Shepherds and Miniature; Australian Stumpy Tail Cattle Dogs; Chesapeake Bay Retrievers; Chinese Cresteds; Cockapoos; Dwarf Poodles; English Cocker Spaniels; Entlebucher Mountain Dogs; Finnish Lapphunds; Goldendoodle; Golden Retrievers; Karelian Bear Dog; Kuvasz; Labradoodles; Labradoodles Australian; Labrador Retrievers; Lapponian Herders; Miniature Poodles; Nova Scotia Duck Tolling Retrievers; Portuguese Water Dogs; Spanish Water Dogs; Swedish Lapphunds; Toy Poodles;
rcd1-PRA		Irish Setters and Irish Red & White Setters; Sloughis;
rcd3-PRA		Cardigan Welsh Corgis
Type A-PRA		Miniature Schnauzers
XL - PRA		Siberian Huskies
X-SCID	X-linked Severe Immunodeficiency	Basset hound , Welsh Corgi .
vWD I	von Willebrand disease Type I	Scottish Terrier , Shetland Sheepdog (Sheltie) .
vWD II	von-Willebrand disease type II	Doberman , German Pinscher , Manchester Terrier , Pembroke Welsh Corgi ,
vWD III	von Willebrand disease Type III	German Wirehaired Pointer

The Dept of Animal Science – Spin off Vetogene patented a DNA test for carrier identification of Junctional Epidermolysis Bullosa in German Pointer. Canine Junctional Epidermolysis Bullosa (JEB) is an inherited autosomal recessive disease, which expression is characterized by skin blistering in different body regions. The disease is clinically characterized by a bullous and ulcerative dermatitis.

The disease causes a general fragility of the skin leading to the formation of the spontaneous or traumatically induced blisters and ulcers on footpads, ear surface, tail tips and pressure points on the distal limbs. The German Shorthaired Pointer (Kurzhaar) is predisposed. The DNA test allows the molecular identification of healthy carriers of JEB by detection of the genetic mutation in the laminin 5 subunit gene (LAMA 3) (17, 18, 19).

As in all the autosomal recessive traits, the meaning of the DNA test provided by the Laboratory is as follows:

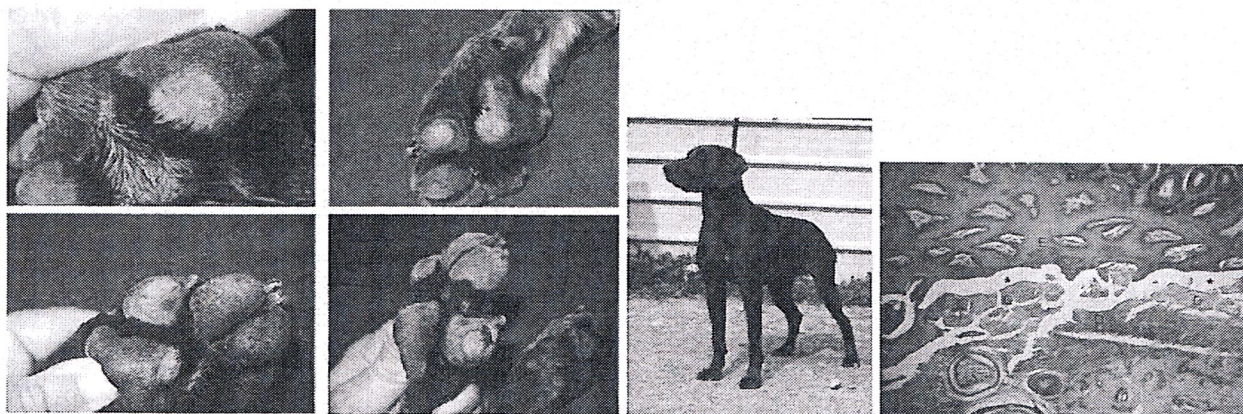
-/- is negative - The subject does not possess the physical attribute and/or disease being tested and therefore will not pass it to its offspring.



+/- is a carrier - The subject carries the physical attribute and/or disease being tested and has one normal gene and one affected gene and therefore will pass the trait to 50% of its offspring.

+/+ is positive - The subject carries the physical attribute and/or disease being tested and has two affected genes and therefore will pass the trait to 100% of its offspring.

Fig1. Ulcers in footpads caused by JEB; German pointer; histological aspect of JEB lesions



### Cat

In domestic cat DNA-based test are available for few inherited defects diseases, so far, see Tab2

Table 2

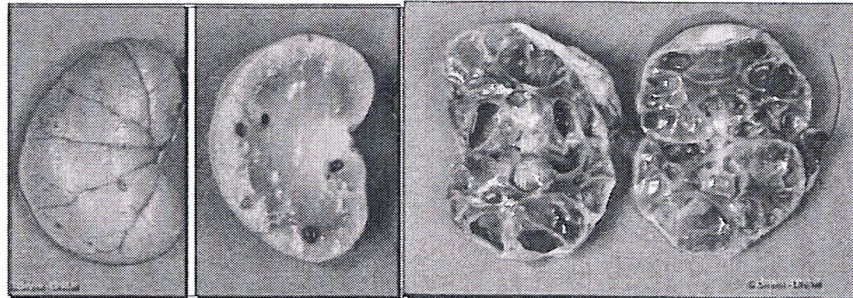
Acronym	Defect	Breed
BLDAB - B	B Blood Group- neonatal isoerythrolysis	All breeds
G1/G2	Gangliosidosis G1 / G2	Korat , Siamese
GSD	Glycogen Storage Disease Type IV	Norwegian Forest Cats
HCM	Hypertrophic cardiomyopathy	Main Coon, Ragdoll
MPS1 and MPSM	Mucopolysaccharidosis SEVERE and MILD form	Siamese
PK	Pyruvate kinase deficiency	Abyssinians, Somalis, DSH
PKD	Feline Polycystic Kidney Disease	Persian and Persian related
rdAc-PRA	Progressive Retinal Atrophy	Abyssinians, Somalis

The two most important defects are PKD and HCM. Both of them are autosomal dominant, highly prevalent (>30%) in cosmopolitan breeds (20). PKD affects Persian, Himalayan, and Exotic cats. Characterized by the formation of cysts on the kidneys (Fig.2), PKD could lead to eventual renal failure and possible death. Echography can detect few small cysts in early age only when performed by specialists in perfect technical conditions, while DNA test always spots the inherited form also before 2 month of age. PKD is dominant; therefore, heterozygotes (those possessing one copy of the mutation) will likely be clinically affected. When bred to



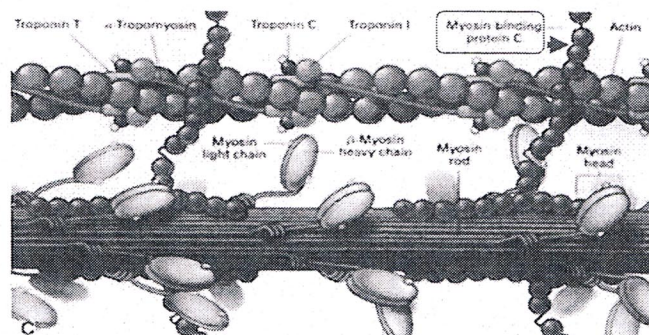
genotypically normal cats, heterozygotes will still likely produce clinically affected offspring 50% of the time.

Fig 2. Kidney of cats with mild and severe PKD (Courtesy Prof. G.Sironi, UNIMI)



The second most important inherited defect in purebred cats is HCM. It is one of the most common causes of sudden cardiac death in young adults and is a familial disease in at least 60% of cases, whether they are random bred or pedigreed. HCM is often a progressive disease with autosomal dominant mode of inheritance. Affected cats usually do not have phenotypic evidence of HCM before 6 months of age, developed HCM during adolescence, and developed severe HCM during young adulthood. Papillary muscle hypertrophy that produced midcavitary obstruction and systolic anterior motion of the mitral valve is the most consistent manifestation of HCM. Cats die suddenly or of heart failure. Histopathology of the myocardium revealed myocardial fiber disarray, intramural coronary arteriosclerosis, and interstitial fibrosis. Two autosomal dominant causative mutations in the feline myosin binding protein C (MYBPC3) gene that computationally alter the protein conformation of this gene, the sarcomeric organization and result in the development of familial HCM have been identified, one in Main Coons and a different one in Ragdolls (21, 22).

Fig.3 Sarcomeric proteins



However only a proportion of genetically positive affected cats develop heart failure if the muscle hypertrophy and subsequent scarring of the heart muscle significantly affects heart function, pointing out the pathogenetic process is still poorly understood and possibly more genes are involved. Additional studies are indicated to explore the relationship between genotype and clinical outcome in affected cats. Moreover because of the high prevalence of this mutation, a breeding



recommendation to eliminate all cats with the mutation could have a substantial impact on the gene pool.

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