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A web resource on DNA tests for canine and feline hereditary diseases

Jeffrey Slutsky [a], Karthik Raj [a], Scott Yuhnke [a], Jerold Bell [b], Neale Fretwell [c], Ake Hedhammar [d], Claire Wade [e], Urs Giger [a,*]

[a] School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA
[b] Department of Clinical Sciences, Tufts Cummings School of Veterinary Medicine, North Grafton, MA, USA
[c] UK Waltham Centre for Pet Nutrition, Freeby Lane, Fretwell, Leicestershire, UK
[d] Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden
[e] Faculty of Veterinary Science, University of Sydney, New South Wales 2006, Australia

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ABSTRACT

Following the first identification of a disease-causing mutation in dogs in 1989 and the more recent completion of canine and feline genome sequences, much progress has been made in the molecular characterization of hereditary diseases in dogs and cats. To increase access to information on diagnosing hereditary diseases in dogs and cats, a web application has been developed to collect, organize and display information on available DNA tests and other supporting information, including gene and chromosomal locations, mutations, primary research citations and disease descriptions. The DNA testing information can be accessed at the URL: http://research.vet.upenn.edu/WSAVA-LabSearch. There are currently 131 molecular genetic tests available for hereditary diseases in dogs and cats offered by 43 laboratories worldwide. This tool should provide clinicians, researchers, breeders and companion animal owners with a single comprehensive, up-to-date and readily searchable webpage for information on hereditary disease testing.

Introduction

Next to humans, the largest number of naturally occurring hereditary disorders and genetic predispositions to disease has been reported in dogs (Sargan, 2003; Giger et al., 2006; Bell et al., 2012), followed by cats (Giger and Haskins, 2006; Pontius et al., 2007; Lyons, 2010, 2012). Notably, many hereditary disorders in dogs and cats represent true homologues of genetic diseases in humans and thus serve as valuable naturally occurring disease models (Marschall and Distl, 2010; Mellersh, 2011). Since many of these disorders are recessively inherited and occur with high frequency in specific or related breeds due to common inbreeding practices, they represent a serious health problem for companion animals (Padgett, 1998; Vella et al., 1999; Giger et al., 2006; Asher, 2009; Hedhammar and Indrebø, 2011; Bell et al., 2012). To address this issue, a thorough investigation of hereditary disorders, from clinicopathologic features to the molecular genetic basis of disease, has become a high priority.

Much progress has been made in the molecular characterization of hereditary diseases in dogs and cats since the initial identification of the genetic basis for canine hemophilia B in 1989 (Evans et al., 1989), aided by the completion of the canine (Lindblah-Toh et al., 2005) and feline (Pontius et al., 2007) genome sequences, and their recent improved coverages and annotations (National Center for Biotechnology Information, NCBI).[1] Thus far, most of the characterized hereditary disorders involve single gene defects with simple Mendelian inheritance and are mostly breed specific (Giger and Haskins, 2006; Giger et al., 2006).

Knowing the specific molecular defect for a hereditary disease is valuable, since it offers the best opportunity to make a precise diagnosis for an animal with clinical signs, helps to screen animals at risk of developing the disease, permits identification of carrier animals (heterozygous for a mutant allele but clinically healthy) and can be used to test animals prior to breeding to assure that affected animals are not produced in future generations (Giger et al., 2006; Lyons, 2010; Mellersh, 2011). The original research laboratories where a disease-specific mutation is first discovered in a particular breed may or may not continue testing animals subsequent to the completion of the relevant research. However, other university or for-profit laboratories may offer these tests following the publication of the mutation, depending on patent and licensure restrictions. The extent of information that is provided to the public varies from one testing laboratory to another, but usually comprehensive information on either the disease or mutation is unavailable.

* Corresponding author. Tel.: +1 215 8988830.
E-mail address: giger@vet.upenn.edu (U. Giger).

[1] See: http://www.ncbi.nlm.nih.gov/.

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Table 1
Sources of genetic disease information.

OMIA	University of Sydney	http://omia.angis.org.au/home; http://www.ncbi.nlm.nih.gov/omia/?term=omia
CIDD	University of Prince Edward Island	www.upei.ca/cidd
LIDA	University of Sydney	www.sydney.edu.au/vetscience/lida
IDID	Cambridge University	http://server.vet.cam.ac.uk
OFA	Orthopedic Foundation of America	http://www.offa.org
CHF	Canine Health Foundation	http://www.akcchf.org
Fabcats	Feline Advisory Bureau	http://www.fabcats.org/breeders/inherited_disorders

It is often daunting for veterinary clinicians, breeders and researchers to keep up with rapid advances in diagnostic opportunities. Despite a number of sources of genetic disease information currently available on-line (Table 1), a few books (Bell et al., 2012), book chapters, review articles and websites that have attempted to gather information on genetic disease testing laboratories, the number of disease-associated mutations, tests offered and laboratories involved continue to grow and change, rapidly rendering many of these sources obsolete (Nicholas et al., 2011; Mellersh, 2012).

To provide a comprehensive resource to find up-to-date, verified information on the currently available DNA tests for inherited diseases in dogs and cats, the Hereditary Disease Committee of the World Small Animal Veterinary Association (WSAVA) has developed a web application featuring an interface that allows users to search the underlying database, which we describe below.

Materials and methods

The Canine and Feline Hereditary Disease (DNA) Testing Laboratories[2] web application was developed using Microsoft ASP.net and a Microsoft SQL server database. The pages and database for this application are hosted on servers at the School of Veterinary Medicine of the University of Pennsylvania (PennGen).

We screened the scientific literature for the molecular characterization of hereditary diseases and genetic predispositions to disease in dogs and cats using PubMed[3] and Commonwealth Agricultural Bureau (CAB) Abstracts.[4] We also searched the Internet for laboratories that offer DNA testing for genetic diseases in dogs and cats. We further checked the availability of DNA tests with dog and cat fancier associations, e.g. American Kennel Club (AKC), The Kennel Club (KC) UK, Fédération Cynologique Internationale (FCI), Cat Fancier Association (CFA) and The International Cat Association (TICA), and organizations involved with genetic health issues in dogs or cats, e.g. Canine Health Foundation (CHF), Orthopedic Foundation of Animals (OFA) and Winn Feline Foundation. Each laboratory was contacted directly and asked for specific information on each test, including which mutation(s) the laboratory tests for, which species and breeds are affected by each mutation tested for, if testing is still available for each DNA test and if additional DNA tests are offered.

In addition to reviewing the published studies and research abstracts in which mutations were first described, we also verified unpublished information with research laboratories to identify additional disease-causing mutations and/or breeds affected by the same or different mutations in the same gene for which tests are now offered. The veracity of all unpublished information has not been verified by the authors, but generally the information is from established laboratories. Genetic information regarding the diseases listed, including gene affected, chromosome and mutation description, was obtained mainly through original research papers and published research on NCBI and PubMed. Mutations were described using the standard nomenclature as described by the Human Genome Variation Society.[5] In addition, genome and other databases in NCBI and the Genome Annotation Resource Fields – *Felis catus* (GARField) in the National Cancer Institute's Laboratory of Genomic Diversity (Pontius and O'Brien, 2007)[6] were used to describe the chromosomal loci of the genes in dogs and cats, respectively.

In some cases, the mutation in the database may be listed slightly differently to that in the published literature due to new information on gene structure, release of updated genome assemblies, use of non-standard nomenclature and occasional errors in mutation descriptions. Online Mendelian Inheritance in Animals (OMIA) and Online Mendelian Inheritance in Man (OMIM) numbers were collected from their websites or based on information provided by laboratory responses. During the analysis, it became evident that the NCBI used a different numbering system than OMIA for trait IDs, which caused confusion; fortunately, this has been corrected by NCBI following consultation. Descriptions on each hereditary disease are continuously being collected from the Veterinary Information Network (VIN) Associate ebook for Hereditary Diseases.[7]

Table 2
Information available in the Canine and Feline Hereditary Disease (DNA) Testing Laboratories web application.

Disease information	Genetic information	Laboratory information
Disease name	Chromosome	Laboratory name
Related terms/synonyms	Gene	Website URL (hyperlink)
Commonly used code	Mutation description	*E-mail* contact
OMIA/OMIM number	Research citation	Mailing address
Breeds affected	Research hyperlink	Country
Clinical disease description		

For the purposes of the data contained in this application, we defined a single heritable disease as an illness characterized by typical signs and routine laboratory tests and/or imaging abnormalities that occur due to a mutation in a particular gene. Therefore, if two breeds present with similar disease phenotypes, but differ in the gene mutated, the resulting disorders would be classified as separate diseases. However, in the case where there are distinct mutations in the same gene in different breeds, causing the same illness, all these mutations would be listed as the same disease.

Only dog breeds recognized by the AKC, FCI and KC were included in the database and we have not included information on mixed breeds unless they uniquely express a specific mutation not seen in any purebreds. Any disease seen in a purebred dog or cat can, of course, occur in a mixed breed animal. For cats, we have included domestic shorthair and domestic longhair cats as their own 'breeds', along with the standard pure breeds, as stated by CFA and TICA. Since our data focuses on disease-specific mutations, tests for parentage and coat color, length and texture are excluded, unless directly associated with a disease. Finally, inclusion of affected breeds was limited to those backed by specific research, although on certain occasions we have allowed a broader interpretation, where the mutation has been found through testing, but not confirmed in a published original study. No DNA mutation screen panels are included in the data.

Results

The verified information on available DNA tests for hereditary diseases and genetic predispositions to diseases in dogs and cats is displayed on a website.[8] We summarize here the information contained in the database to mid-2012 (Tables 2–6). It was discovered that four laboratories stopped offering DNA tests during the collection period and are therefore not included in the data. Forty-four laboratories offered DNA tests for hereditary diseases in dogs and cats, 43 of which were included in the database and whose data we report on below; one corporate laboratory requested to be excluded from the database. The name, address and website for each laboratory, as well as details of each DNA test are provided. Twenty-two of the 43 testing sites are the laboratories and/or the investigators that originally identified the mutation. These usually only test for a single mutation or a small group of (related) genetic diseases; 14 laboratories only test for a single disease and nine of these only test samples from a single breed bearing the mutation.

[2] See: http://research.vet.upenn.edu/WSAVA-LabSearch.
[3] See: www.ncbi.nlm.nih.gov/pubmed.
[4] See: www.cabi.org.
[5] See: http://www.hgvs.org/mutnomen.
[6] See: http://lgd.abcc.ncifcrf.gov.
[7] See: http://www.vin.com/Members/Associate/Associate.plx?Book=1&BrowseChapter=&SpeciesID=5#Jump.
[8] See: http://research.vet.upenn.edu/WSAVA-LabSearch.

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Table 3
Information available from 'View Disease Details' link.

Disease name/synonyms
General description
Description in species
Mode of inheritance
Etiology
Breed, sex and age predilection
Clinical findings and signs
Diagnostic procedures
Treatment and management
Prevention
Differential diagnosis
Human disease homologue
Available tests
Research references
Contributor's name and date

Of 43 laboratories that offered DNA testing, 21 were commercial laboratories that specialize in genetic disease testing. Twenty-eight laboratories offered DNA tests for dogs only, five for cats only and 10 for dogs and cats. No laboratory offers all available tests, due to restrictions by patents, limited licensure, through a specific disease focus of the laboratories and/or through a lack of demand to test for mutations that occur very rarely in a particular breed population (Table 5).

Table 6
Inheritance patterns of diseases with known mutations.

	Dogs	Cats	Total
Autosomal recessive	107	19	126
Autosomal dominant	13	4	17
X-linked recessive	1	2	3
X-linked dominant	8	0	8
Mitochondrial	1	0	1

A total of 155 hereditary diseases (130 in dogs, 25 in cats) have been characterized at the molecular level and 125 currently can be assessed in laboratories (111 in dogs, 20 in cats). Although 94 disorders can be tested for by several laboratories (85 in dogs, 9 in cats), the rest are offered only by a single laboratory (Table 4), either due to patent and license restrictions, lack of published information and/or because the mutation is believed to occur very rarely in a particular breed population. More than one mutation has been reported in the same gene for several disorders

Table 4
Summary of disease information in the database.

	Dog	Cat	Total
Number of disease tests	111	20	125[a]
Diseases with a single mutation	87	15	102
Diseases with multiple mutations	24	5	29
Total mutations tested for	143	24	167
Single breed mutations	100	15	115
Mutations affecting multiple breeds	43	9	52
Total breed specific tests tested for[b]	361[c]	56[d]	417
Commercial breed specific tests	306	41	347
Non-profit breed specific test	176	35	211
Breed tests available at only one laboratory	123	13	136
Breed tests available at multiple laboratories	238	43	281
Maximum number of laboratories performing a test	10[e]	10[f]	
Maximum number of mutations in a single disease	6[g]	2[h]	
Maximum number of breeds tested for a single mutation	22[i]	16[j]	
Average number of laboratories testing a single breed specific mutation	3.6	3.0	
Median number of laboratories	3	1	
Average number of mutations for a specific disease	1.3	1.4	
Median number of mutations	1	1	
Average number of breeds for a specific mutation	2.3	2.9	
Median number of breeds	1	1	

[a] Includes six diseases where the mutation has been found in both species and a test is available in both species.
[b] Total of the tests for each specific mutation available in a specific breed (i.e. a specific disease/mutation/breed combination).
[c] There are 121 breed specific tests for dogs available at both commercial and non-profit laboratories.
[d] There are 20 breed specific tests for cats available at both commercial and non-profit laboratories.
[e] Multiple instances.
[f] Blood type B mutation.
[g] Factor IX deficiency (hemophilia B).
[h] Multiple instances.
[i] Primary lens luxation.
[j] Progressive retinal atrophy (*Rdac* mutation), although Blood type B is offered for all breeds.

Table 5
Summary of laboratory information in the database.

	Non-profit	Corporate	Total
Number of laboratories	22	21	43
Average number of diseases tested by one laboratory	5.0	20.0	12.4
Median number of diseases tested by one laboratory	2	15	4.0
Maximum number of diseases tested by one laboratory	27	67	
Minimum number of diseases tested by one laboratory	1	1	
Average number of breed mutation tests by one laboratory	13.5	57.2	34.8
Median number of breed mutation tests by one laboratory	4.5	47	
Maximum number of breed mutation tests by one laboratory	60	195	
Minimum number of breed mutation tests by one laboratory	1	1	

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(24 disorders in dogs, five in cats); frequently, individual mutations are breed specific. The pattern of inheritance of the majority of diseases in dogs and cats with known mutations is autosomal recessive; mutations that are inherited as autosomal dominant, X-linked recessive, X-linked dominant or mitochondrial traits have also been identified (Table 6). Tests for several complex traits with multiple gene defects need to be investigated further.

Many mutations were found only in a single breed (69% of the mutations listed in the database), whereas some mutations have been found in multiple breeds, up to 22 for primary lens luxation. Some disorders have only been identified in a single animal or family and may not be present in the general breed population, e.g. X-linked severe combined immunodeficiency in dogs maintained in a research colony (Henthorn et al., 1994); routine testing for such

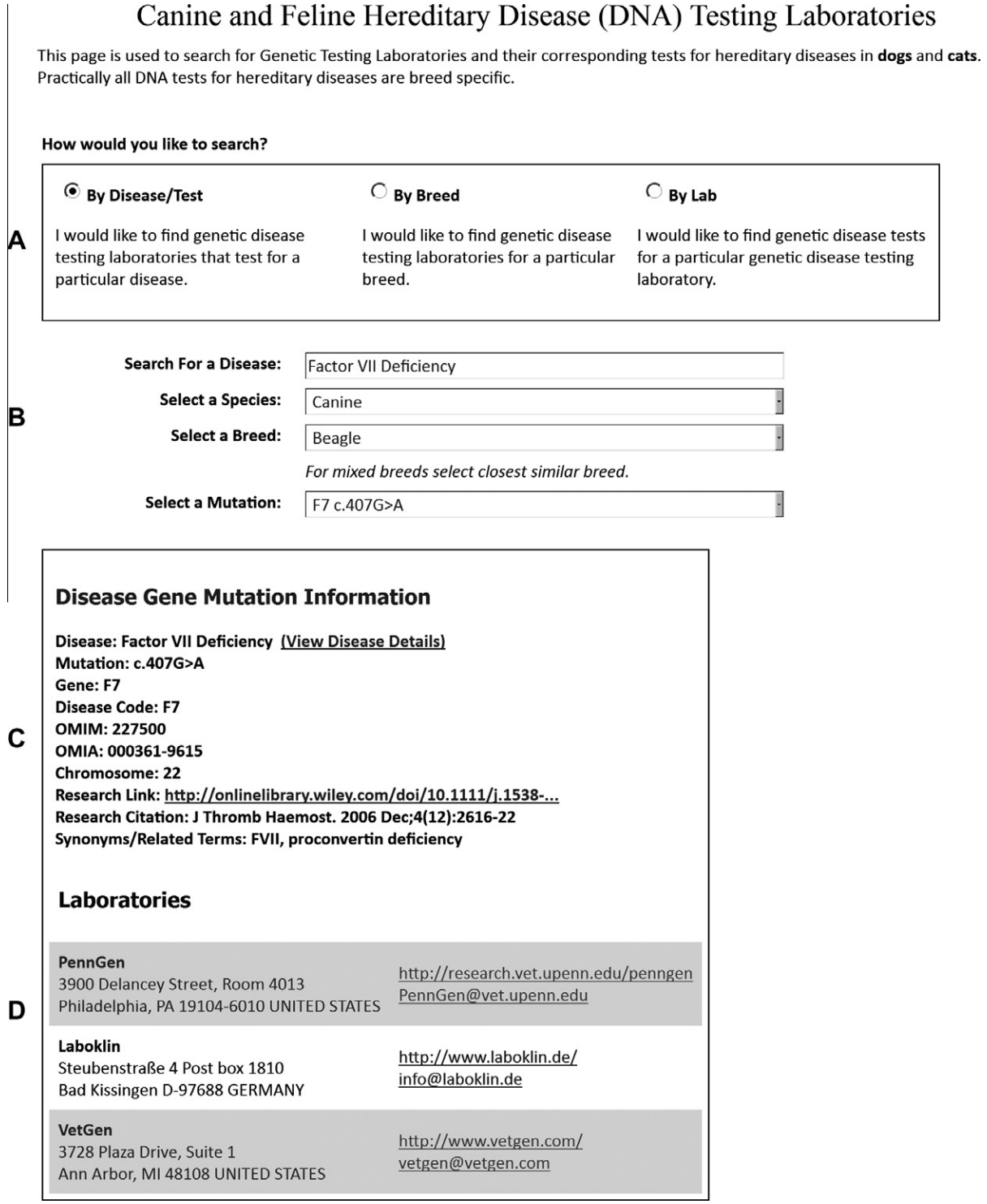

Fig. 1. A sample disease test search for a coagulopathy in Beagles. (A) Searches can be done by disease/test, breed or laboratory. (B) Information regarding the selection is used to narrow down the results. (C) Information about the specific disease in this breed is displayed. (D) Information about the laboratories doing the specific test in this breed is displayed.

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specific mutations usually is not offered. There are also cases where there are separate mutations affecting the same breed, causing different forms of the disease, e.g. porphyria in domestic shorthair cats (Clavero et al., 2010).

Discussion

In the past two decades, much progress has been made in the characterization of disease-causing mutations in dogs and cats. Through DNA testing, this new information permits specific diagnosis in an animal affected by a specific hereditary disease or allows an animal at risk of becoming ill because of a particular disease-causing mutation to be identified. Most genetic diseases are inherited recessively and may occur commonly in one or more breeds due to particular breeding practices, such as deliberate inbreeding or the extensive use of a popular sire (Wade, 2011). Therefore, knowledge of the mutation allows screening of the breeding stock and, by permitting selection of appropriate breeding animals, can eliminate the disease from future generations.

DNA tests are the most desirable tools for the detection of mutations causing hereditary diseases; they allow determination of homozygosity and heterozygosity for a certain mutant/disease allele, only require small samples (such as blood or cheek swabs, which can be shipped by regular mail), are relatively simple to perform in the laboratory, are standardized and are potentially less expensive than most other tests. There are many different techniques, from manual to robotically automated, for identification of the normal and mutant allele for a disease. This web application does not provide information on these detailed laboratory techniques, which often change with new technologies. Moreover, currently there is no official quality control system for DNA testing in veterinary medicine and the application presented here cannot assess the quality of testing of any laboratory listed.

Although biochemical laboratory tests and imaging studies are used to diagnose some hereditary diseases in companion animals, genomic DNA tests for single gene defects are considered to be the most accurate in clinical medicine and thus only DNA tests are included here. Allowing for human errors from identifying animals, labeling and mixing up samples, these DNA tests are considered to be accurate, assuming that regular laboratory standards, with appropriate positive and negative controls, are followed.

Current information on mutant allele frequency is limited, since the data generally are based upon a few rather small and frequently biased, rather than randomized, surveys or open registries. Also, common mutations may disappear from a population (breed) due to the success of a DNA screening program.

Recently, one company involved in canine disease testing has offered a multiple single nucleotide polymorphism (SNP) panel analysis that screens for disease-causing mutations in mixed breed dogs (Mars Veterinary). This company was not included on the website, since panel analysis screens are not considered to be a specific breed test. The results of the panel are not reported as a definitive diagnosis in affected animals, but alert the submitter if a mutation is found, so that further specific testing can be pursued at a DNA genetic disease testing laboratory. Unless patents and licensures restrict its future use, such panel analyses may be used for all known DNA mutations in a species, making this method a simple and cost effective tool to screen for hereditary diseases in companion animals.

Our website is arranged by general categories: disease, breed, and laboratory, each of which can be searched separately (Fig. 1A). After selecting an initial category to search, the users may select the specific disease, species (canine/feline) and breed they are interested in. If there is more than one mutation known to cause a disease, the specific mutation can be selected. As an example, we have chosen to search for factor VII deficiency, a common coagulopathy (Callan et al., 2006) (Fig. 1B). The application displays the pertinent genetic information regarding the hereditary disease (Fig. 1C), as well as the laboratories that offer the test (Fig. 1D). If further clinical details on the disease are desired, they may be accessed via the hyperlink through the 'View Disease Details' option to download a PDF file (Fig. 1C; Table 3).

In the example shown in Fig. 1, three testing laboratories are identified. The first laboratory listed will be the laboratory that originally identified the particular breed-specific disease mutation, if they are still testing for the mutation, or a laboratory that is directly affiliated with the research group. The research article first describing the mutation may be accessed (Fig. 1C) through the textual citation or through a hyperlink (in this case freely accessible by the hyperlink to PubMed Central). This disease example also reveals that two other breeds have Factor VII deficiency caused by the same mutation (Alaskan Klee Kai and Scottish deerhound). While this coagulopathy has also been described in Great Pyrenees and English springer spaniels, the disease-causing mutation(s) in these breeds have not yet been identified. Since the DNA test may not be helpful for these and other breeds, currently they are not contained in the database under this mutation test.

Conclusions

This web-based application represents a source of up-to-date information on hereditary diseases in companion animals for veterinary clinicians looking for a laboratory to perform a test, researchers searching for information on hereditary diseases and owners/breeders with affected animals or animals at risk of developing a particular disease or passing on the mutant allele (carriers). We intend to keep this web application updated by regular review of the pertinent literature, correspondence with testing laboratories and through feedback from those involved in research on comparative medical genetics. This service will be continued by the WSAVA Hereditary Disease Committee.

Conflict of interest statement

The authors from the University of Pennsylvania are associated with PennGen, one of the not-for-profit laboratories offering DNA tests, and the work was funded by the WSAVA through contributions from Waltham.

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