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

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In 2012 scientists at the Kennel Club Genetics Centre at the Animal Health Trust undertook a study to measure the frequency of the mutations responsible for congenital keratoconjunctivitis sicca and ichthyosiform dermatosis (dry eye and curly coat syndrome) and episodic falling in Cavalier King Charles Spaniels in the UK. This report describes the study, its conclusions and recommendations for breeders

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Introduction

The Kennel Club Genetics Centre at the Animal Health Trust (AHT) carries out research aimed at understanding and eradicating inherited diseases in purebred dogs. Our research over the last twenty years has greatly advanced our knowledge of inherited canine disorders, and the DNA tests we have developed have helped countless dog breeders improve the genetic health of their dogs.

New DNA Tests

In April 2011 the AHT was pleased to announce the launch of two new DNA tests for Cavalier King Charles Spaniels, both of which were developed by researchers at the Kennel Club Genetics Centre at the AHT. Mutations in the DNA of two separate genes were identified[reference]. The first mutation causes episodic falling **(Box 1)**, an inherited disease that affects the nervous system. The second mutation is responsible for a condition known as dry eye and curly coat syndrome (or simply "curly coat syndrome") **(Box 2)** but it also has the more formal name of congenital keratoconjunctivitis sicca and ichthyosiform dermatosis. Both of these conditions are inherited in a **recessive** manner.

Basic Genetics

Every dog inherits two sets of genes - one set from each of its parents. Most Cavaliers will inherit two normal copies of the genes associated with episodic falling and curly coat syndrome. These dogs are **clear** of the mutations. For a small number of puppies, both copies of one or other of these genes are mutated, and they will be born **affected** by episodic falling or curly coat syndrome.

Some dogs inherit one normal copy and one mutated copy of a gene. These dogs are called **carriers**. They show no signs of the recessively inherited disease themselves, but if they are mated to another carrier some of the puppies may be born with two copies of the mutation (one from each parent) and be affected by the disease. By taking simple cheek swabs from their dogs and sending them to the AHT for DNA testing, Cavalier breeders can use the genotyping results to identify which of their dogs are carriers of episodic falling and/or curly coat syndrome. If a carrier is mated to a partner who is clear of the same mutation, the pair will not produce any puppies affected by that disease. It is worth remembering episodic falling and curly coat syndrome are caused by different mutations that are independent from one another, so a dog can carry zero, one or two copies of either mutation.

Details of Study

A DNA test can tell us whether a dog is clear, affected or a carrier. Nobody can tell whether a Cavalier is clear or a carrier of episodic falling or curly coat syndrome just by looking at it. This makes it very difficult to know what percentage of dogs are carrying the episodic falling and curly coat syndrome mutations.

Researchers at the Kennel Club Genetics Centre set out to calculate the frequencies of the episodic falling and curly coat syndrome mutations in the UK Cavalier population by collecting and testing DNA from a wide selection of dogs of breeding age. Finding out how many carriers there are allows us to customise the guidance we offer to Cavalier breeders on breeding strategies and the best way to work towards the elimination of these inherited conditions from the breed. The results also provide a useful benchmark against which progress can be monitored over the next few years.

Box 1: Clinical signs of episodic falling

- usual age of onset 3-7 months old
- brought on by excitement, exercise or stress
- sudden increase in muscle tone and inability to relax muscles
- abnormal postures or gait, often followed by collapse forwards or to the side
- episodes are usually brief, lasting a few seconds to several minutes
- no loss of consciousness

Box 2: Clinical signs of dry eye and curly coat syndrome ("curly coat syndrome")

- congenital (present at birth)
- affected puppies are often smaller in size than littermates
- eyes become sore and weepy soon after eyelid opening at 10-14 days
- curly, crimped or rough appearance to coat which can later become sparse
- itchy, scaly skin
- hard, thickened footpads that often crack and become sore
- deformed nails that occasionally fall out, causing pain and lameness

Recessive: a condition that appears only in dogs who have received two copies of a mutant gene, one copy from each parent. The individuals with a double dose of the mutated gene are called homozygotes

Affected: these dogs have inherited two copies of the mutation and usually show clinical signs of the disease. They are homozygous, and if bred from they will pass a copy of the mutation to 100% of their offspring

Clear: these dogs are also homozygous but have inherited two normal copies of the gene and show no signs of the disease. They will not pass on the mutation to their offspring

Carrier: these dogs have inherited one normal copy and one mutated copy of the gene, show no clinical signs of the disease and are called heterozygotes. On average they will pass on a copy of the mutation to 50% of their offspring

Reference: Parallel mapping and simultaneous sequencing reveals deletions in *BCAN* and *FAM83H* associated with discrete inherited disorders in a domestic dog breed. Forman *et al* (2012) http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1002462

Box 3: Eligibility criteria for participants

All participants:

- Cavalier King Charles Spaniels registered with the Kennel Club in the UK

Breed Club set:

- all dogs within set to have a unique dam
- no more than four dogs within the set to share a sire
- date of birth not before 1st July 2005
  (exception made for older males still available at stud)

Random set:

- transfer of ownership recorded in Breed Records Supplements 2008-2010
- date of birth not before 1st July 2005

Study participants

Many Cavalier owners in the UK are members of one or more of several breed-specific clubs recognised by the **Kennel Club** (KC). The KC encourages breed clubs to address any health issues within the breeds they represent. Each breed appoints a health representative responsible for liaison between the KC and the individual Breed Clubs.

Our researchers asked Peter Towse, the health representative for Cavaliers, for his assistance in recruiting candidates for our study. He was very willing to help and quickly received the support of officials from all eleven of the Cavalier clubs recognised by the KC. Ten of these clubs are regional in nature and it was proposed that each of these regional clubs would put forward the names of twenty dogs as candidates for the study, making a total of 200 dogs drawn from across the UK. During the period of the study a new Cavalier club applied for KC recognition. The research team was pleased to invite this club to participate alongside the regional clubs. Eligibility criteria are listed in **Box 3**.

Breed Club officials have long observed that some 80% of puppies in this popular breed are registered by breeders and other individuals who are not members of the KC recognised clubs. This pool of breeders is said to include "commercial" kennels and "hobby" breeders who do not hold a KC approved **Kennel Name**. The research team felt that some attempt should be made to obtain samples from this additional pool. We randomly selected 400 Cavaliers from 17,016 "transfers of ownership" recorded in the KC **Breed Records Supplements** for 2008, 2009 and 2010. With the help of Prof Jeff Sampson and the staff at the KC, the owners of these 400 dogs were also sent invitations to take part in the study.

Owners were sent a DNA sampling kit for each participating dog and asked to collect cheek cells using the buccal brushes provided. To simplify the sampling process as much as possible, there was no requirement for the identity of any dogs in the study to be verified by a veterinary surgeon.

Samples received

We received 285 samples in total, of which 280 were eligible for inclusion in the study. A breakdown of submissions by age, sex and colour can be found in **Table 1**. Contributions were received from all regions of the UK.

Table 1: Age, sex and colour of participating dogs

	no of dogs	age	sex		colour			
		median	male	female	blen	tri	ruby	b/t
Breed Club set	154	3.7 yrs	34%	66%	53%	22%	11%	14%
Random set	126	3.2 yrs	37%	63%	37%	26%	19%	18%
All participants	280	3.4 yrs	35%	65%	46%	24%	14%	16%

Abbreviations: blen = blenheim, tri = tricolour, b/t = black and tan
All percentages shown have been rounded up to one decimal place and may not add up to exactly 100%

Kennel Club: a large organisation in the UK founded in 1873 with the aim of "promoting in every way the general improvement of dogs". Provides a registration service for pedigree and crossbred dogs and runs the Assured Breeder Scheme

Kennel Name: more commonly known as an "affix". A unique word approved by the Kennel Club for exclusive use by a breeder when naming puppies

Breed Records Supplement: a quarterly publication recording all registration-related applications made to the Kennel Club

Breed Club samples

Samples submitted by owners enlisted with the help of Breed Club health representatives accounted for 154 (55%) of the total. Members of nine of the ten regional Breed Clubs took part, along with members from the national club and the newly-established club. Examination of the registered names of all 154 dogs from the Breed Clubs revealed that 95% were prefixed with a KC approved Kennel Name. All 154 dogs in this set had a unique dam, and 129 individual sires were represented. Of these sires, 21 were represented by two, three or four progeny.

Random samples

From the 400 invitations sent out at random with the help of the KC, 126 owners chose to participate. This represented a response rate of 31.5%, and accounted for 45% of the total number of dogs in the study. Fewer of the registered names of these dogs (54%) were prefixed with a KC approved Kennel Name. The parentage of the dogs in this randomly selected set was not restricted, but analysis of registration details revealed that 113 unique sires and 119 unique dams were represented within this set.

Results

Mutation frequencies

We calculated the episodic falling and curly coat syndrome **mutation frequencies** by counting how many of the **chromosomes** in the dogs we sampled carried mutant **alleles (Tables 2 & 3)**. Our calculations show that the mutation frequency for episodic falling is **0.11**, and for curly coat syndrome it is **0.06**. There was no statistically significant difference between the Breed Club set and the random set for either mutation.

Mutation frequency: refers to the number or "frequency" of chromosomes with a specific mutation in a given population at a given point in time

Chromosome: a threadlike structure of DNA and protein found in the nucleus of most living cells that carry the genes in a linear order. All animals have two copies of each chromosome

Allele: one of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome

Table 2: Episodic falling: allele frequencies

	number of dogs				normal allele	mutant allele
	clear	carrier	affected	total	p	q
Breed Club set	127	24	3	154	0.90	0.10
Random set	97	28	1	126	0.88	0.12
All participants	224	52	4	280	0.89	**0.11**

Table 3: Curly coat syndrome: allele frequencies

	number of dogs				normal allele	mutant allele
	clear	carrier	affected	total	p	q
Breed Club set	133	21	0	154	0.93	0.07
Random set	115	11	0	126	0.96	0.04
All participants	248	32	0	280	0.94	**0.06**

Table 4: Episodic falling: allele frequencies extrapolated to the whole population

	normal allele	mutant allele	clear	carrier	affected
	p	q	p^2	2pq	q^2
Breed Club set	0.90	0.10	81.5%	17.6%	0.9%
Random set	0.88	0.12	77.6%	21.0%	1.4%
All participants	0.89	0.11	79.7%	**19.1%**	1.1%

Table 5: Curly coat syndrome: allele frequencies extrapolated to the whole population

	normal allele	mutant allele	clear	carrier	affected
	p	q	p^2	2pq	q^2
Breed Club set	0.93	0.07	86.8%	12.7%	0.5%
Random set	0.96	0.04	91.5%	8.3%	0.2%
All participants	0.94	0.06	88.9%	**10.8%**	0.3%

All percentages shown have been rounded up to one decimal place and may not add up to exactly 100%

If we make the assumption that the dogs we sampled represent a random subset of the population, we can use the results from our study to extrapolate to the whole of the UK population. If we take it that mating between dogs occurs randomly with respect to these mutations, we use the **Hardy-Weinberg equilibrium** equation to estimate the percentages of clear, carrier and affected dogs in the whole population. Using the letters p and q to represent the normal and mutant alleles respectively, we can apply the equation $p^2 + 2pq + q^2 = 1$ to our frequencies **(Tables 4 & 5 above)**. We are particularly interested in finding out the percentage of carriers in the overall population. Using our calculations we therefore estimate that **19.1%** are carriers of the episodic falling mutation and **10.8%** are carriers of the curly coat syndrome mutation.

We investigated whether the mutation frequencies differed between the four Cavalier coat colours. We found carriers of both mutations in all colours, but it was notable that the episodic falling frequency differed considerably between the "particolours" and the "wholecolours" **(Tables 6 & 7 below)**. The mutation frequency ranged from 0.05 in the blenheims to 0.24 in the rubies. The curly coat syndrome frequency was distributed more evenly between the colours.

Hardy-Weinberg equilibrium: a fundamental principle in population genetics stating that the proportion of clear, carrier and affected individuals within a large, randomly mating population depends on the mutation frequency and remains constant in the absence of immigration and selection

Table 6: Episodic falling: allele frequencies in the four coat colours (280 dogs)

Coat colour	normal allele	mutant allele	clear	carrier	affected
	p	q	p^2	2pq	q^2
Blenheim	0.95	0.05	90.9%	8.9%	0.2%
Tricolour	0.93	0.07	85.6%	13.8%	0.6%
Ruby	0.76	0.24	58.1%	36.2%	5.6%
Black/tan	0.78	0.22	61.5%	33.9%	4.7%
Particolour	0.94	0.06	89.1%	10.6%	0.3%
Wholecolour	0.77	0.23	59.9%	35.0%	5.1%

"Particolour" includes the blenheim and tricolour coat colours; "wholecolour" includes ruby and black/tan
All percentages shown have been rounded up to one decimal place and may not add up to exactly 100%

Table 7: Curly coat syndrome: allele frequencies in the four coat colours (280 dogs)

Coat colour	normal allele	mutant allele	clear	carrier	affected
	p	q	p^2	2pq	q^2
Blenheim	0.93	0.07	85.8%	13.6%	0.5%
Tricolour	0.96	0.04	91.2%	8.6%	0.2%
Ruby	0.94	0.06	87.9%	11.7%	0.4%
Black/tan	0.98	0.02	95.5%	4.4%	0.1%
Particolour	0.94	0.06	87.7%	11.9%	0.4%
Wholecolour	0.95	0.05	89.6%	10.1%	0.3%

Conclusions

Our results show that almost 30% of UK Cavaliers of breeding age are carriers of episodic falling or curly coat syndrome, and a small number are carriers of both. Around 1-2% of Cavaliers carry two copies of the episodic falling and/or the curly coat syndrome mutation and are affected.

Recommendations

- All Cavalier King Charles Spaniels that are to be bred from should be DNA tested for both mutations prior to mating, regardless of colour or ancestry
- When planning a litter, breeders should choose a dog and bitch that cannot combine to produce affected puppies
- Carriers should not be excluded from breeding programmes until the mutation frequency within the breed falls below 0.01 to avoid reducing genetic diversity unduly
- Progress towards elimination of these two inherited diseases from the breed should be monitored by carrying out further mutation frequency checks every few years

See **Appendix 1: Should we breed with carriers?** for further information

Appendix 1
Should we breed with carriers?

"Carrier" is the term given to an individual (of any species) that carries a single copy of a recessive mutation that is associated with a specific inherited condition, usually an inherited disorder. An individual will only suffer from a recessive disorder if it inherits two copies of the causal mutation, one from each parent. If it inherits a single copy of the mutation it will remain healthy but will pass the mutation on to about half of its offspring.

Breeding with carriers

Once a specific disease mutation has been identified, a DNA test can be developed that enables the identification of non-symptomatic carriers. Knowing which dogs carry the mutation and which do not (the so-called "clear" dogs) enables breeders to make sensible choices about the dogs they mate together. All dogs can be safely bred with provided at least one of the mating pair is clear of the mutation (see table below). Breeding dogs that will never develop the condition should obviously be the priority for all conscientious breeders, and the desire to eliminate a disease-associated mutation from a breed should therefore be the long-term goal. But the instinct to choose only clear dogs to breed from, as soon as a DNA test becomes available, may not always be a sensible choice.

If carriers are prevented from breeding, the opportunity to pass the rest of their genetic material to the next generation is also lost and the genetic diversity of the remaining population is thus reduced. It is worth remembering that there is a clear and well-established link between the genetic diversity of a population and its overall health, and that breeding closely-related individuals tends to lead to the accumulation of deleterious recessive mutations in the population. This is due to the fact that an individual is more likely to inherit two identical copies of a mutation if its parents share common ancestors than if they are unrelated, and the more common ancestors the parents share the greater that chance is.

It is also worth remembering that **the disease mutation for which there is a DNA test is not the only mutation a carrier has.** Every human, on average, carries about 50 recessive mutations and there is no reason to believe the average dog will not carry a similar number. So the only real difference between a clear and a carrier is the single mutation that can be tested for. Both dogs will carry around 49 other mutations that the breeder does not know about and cannot test for. If carriers are not bred from and clear dogs are used extensively then there is a real risk that other mutations will increase in frequency in the breed and new inherited disease(s) could emerge.

There is no reason why the eventual elimination of a disease mutation from a breed should not be the goal, once a DNA test for that mutation becomes available. But, providing all breeding dogs are tested for the mutation prior to mating, the breeders can take their time and ensure that desirable traits are not eliminated along with the disease mutation, and that the genetic diversity of the breed is not reduced.

Outcomes of mating combinations

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 50% of puppies will be carriers 25% of puppies will be affected	Yes
Carrier x affected	50% of puppies will be carriers 50% of puppies will be affected	Yes
Affected x affected	All puppies will be affected	Yes

Mutation frequency

The speed with which the mutation can be eliminated depends on several factors, including the frequency of the mutation, the population structure and the rate of inbreeding for that breed. The more frequent the mutation is, the more slowly it should be eliminated. Calculating the true frequency of a mutation is not trivial, and requires a random subset of a breed be screened. Dogs that are tested once a commercial DNA test becomes available are not always representative of the breed as a whole, and similarly cohorts of dogs that have been sampled by a research institute during development of the test are rarely characteristic of the breed.

The frequency of a mutation is typically expressed as the fraction of chromosomes in a population that carry the mutation. For example, if the frequency of a mutation is described as 0.1, this means that 10% of the chromosomes in that breed carry the mutation and the remaining 90% carry the normal copy of DNA. If 10% of the chromosomes carry the mutation then just under 20% of dogs are expected to be carriers and about 1% of dogs will be affected.

Breeding Advice

Carriers should always be included in the first one to two generations that follow the launch of a DNA test for a recessive mutation, regardless of the frequency of the mutation, to give breeders the opportunity to capture desirable traits, such as breed type and temperament, before they start to select for dogs that are clear of the mutation. Specific breeding policy for future generations should be breed-dependent and ideally formulated after consideration of factors such as the population structure and rate of inbreeding. But in general terms, carriers should only be removed from the breeding population if the frequency of the mutation is below 0.01 (1%), as this will mean only around 2% of dogs will be prevented from breeding. Avoiding carriers of a mutation that is more frequent will result in a greater number of dogs being prevented from breeding and could lead to a detrimental loss of diversity for the breed.