



Frequency of the neonatal cerebellar cortical degeneration (NCCD) mutation in Beagles in the UK



"Ash", NCCD clear. Photo courtesy of Sam Goldberg

In 2012 scientists at the Kennel Club Genetics Centre at the Animal Health Trust identified the genetic mutation responsible for a newly-emerging inherited condition in Beagles known as neonatal cerebellar cortical degeneration (NCCD) and launched a DNA test which breeders can use as a tool to inform breeding decisions. This report summarises a study of the frequency of the NCCD mutation in UK Beagles, and makes recommendations for breeders based on its conclusions.

Glossary of terms

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

Mutation frequency: refers to the number or "frequency" of chromosomes with a specific mutation in a given population at a given point in time. For example, if two out of 50 dogs are carriers the mutation frequency is 0.02 or 2%

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

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

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

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

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 test for Beagles

In July 2012 the AHT announced the launch of a DNA test for neonatal cerebellar cortical degeneration (NCCD) in Beagles, following research by scientists at the Kennel Club Genetics Centre at the AHT. NCCD is a rare disease of the nervous system that affects Beagle puppies in the first few weeks of life. Affected puppies cannot walk properly and they fall easily, due to the loss of cells in the brain that are important for correct movement. There is no cure for this condition, and often the kindest option is for these puppies to be put to sleep.

One of our researchers employed cutting-edge techniques to locate the NCCD genetic mutation using samples from a single affected puppy, and a DNA test quickly followed. This condition is inherited

in a **recessive** manner and is not believed to be very common. Sensible use of the DNA test provides an early opportunity to halt the spread of the NCCD mutation through the breed before it becomes firmly established.

Basic genetics

Every dog inherits two sets of genes - one set from each of its parents. Most Beagles inherit two normal copies of the gene associated with NCCD. These dogs are **clear** of the mutation. Sadly for a small number of puppies both copies of these genes are mutated, and they are born **affected** by NCCD.

Some dogs inherit one normal copy and one mutated copy of a gene. These dogs are called **carriers**. They show no signs of the 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 therefore these pups will be affected by the disease. By taking simple cheek swabs and sending them to the AHT for DNA testing, Beagle breeders can use the test results to identify which of their dogs are carriers of NCCD. A carrier can be paired with a partner who is clear of the mutation, and no puppies in the resulting litter will be affected by NCCD.

total number of dogs	age	sex		colour				
		male	female	tricolour	tan & white	lemon & white	tricolour mottle	hare pied
152	median: 3.6 yrs range: 1.3 - 7 yrs	45%	55%	81%	11%	7%	<1%	<1%

Frequency of the mutation

Nobody can tell whether a Beagle is clear or a carrier of the NCCD mutation just by looking at it. This makes it very difficult to know how widespread the mutation is. Now that we have a DNA test available to tell us which dogs are clear and which are carriers, we can use this information immediately to prevent any more Beagle puppies being born that are affected by this unpleasant inherited condition.

To assist breeders in the longer term, researchers at the Kennel Club Genetics Centre set out to calculate the NCCD **mutation frequency** in the UK Beagle population. We did this by collecting and testing DNA from a random selection of dogs of breeding age. Finding out how many carriers there are allows us to customise the guidance we offer to Beagle breeders on breeding strategies and the best way to work towards the elimination of this inherited condition from the breed. The results also provide a useful benchmark against which progress can be monitored over the next few years.

Beagles sampled for the study

The **Kennel Club** (KC) sent out invitation packs on behalf of the AHT to the owners of 500 Beagles selected at random from "transfer of ownership" entries recorded in the **Breed Records Supplement**. To reflect the current breeding population in the UK, selection was restricted to dogs and bitches born between 1st January 2006 and 31st December 2011.

Each pack contained details of the proposed study, brushes for collecting buccal (cheek) cells, a submission form, and a reply paid envelope for return of samples and the completed paperwork to the AHT.

Samples received for DNA testing

From the 500 invitations sent out by the KC, DNA samples from 152 Beagles were sent in to the AHT research team, representing a response rate of 30.4%. Analysis of registration details of the sampled Beagles revealed that 98 unique sires and 139 unique dams were represented. A breakdown of submissions by age, sex and colour can be found in **Table 1**.

DNA test results

We found eight NCCD carriers in our set of 152 Beagles. This gives us a basic figure to start with, but we use a further calculation to provide us with the mutation frequency. Each of the 152 dogs has a pair of **chromosomes** bearing the NCCD gene (one from each parent), meaning that 304 chromosomes are represented in total. We calculate the frequency of the NCCD mutation by counting how many of the chromosomes in the sampled dogs carry the mutant **allele**. The eight carriers each have one normal allele and one mutant allele, so eight out of 304 chromosomes are mutated. We convert this to a decimal number and this gives us the frequency of the NCCD mutant allele (**Table 2**). In our set of Beagles the mutation frequency is **0.03**.

	number of dogs				normal allele	mutant allele
	clear	carrier	affected	total	p	q
Dogs DNA tested	144	8	0	152	0.97	0.03

Allele frequency percentages shown have been rounded up to two decimal places

Table 3: NCCD allele frequencies extrapolated to the whole population

	normal allele	mutant allele	clear	carrier	affected
	p	q	p ²	2pq	q ²
Whole population	0.97	0.03	94.8%	5.1%	0.1%

Clear, carrier and affected percentages shown have been rounded up to one decimal place

Frequency in the overall population

If we make the assumption that the dogs we sampled represent a random subset of the population, we can use the results from our set of dogs to extrapolate to the whole of the UK Beagle population. If we take it that mating between dogs occurs randomly with respect to these mutations, we can use the **Hardy-Weinberg equilibrium** equation to estimate the percentages of clear, carrier and affected dogs in the whole population (**Table 3**). We take the letters p and q to represent the normal and mutant alleles respectively, and then apply the equation $p^2 + 2pq + q^2 = 1$ to our frequencies.

We are particularly interested in the percentage of carriers in the overall population as this will help to guide future breeding strategies. Using our calculations we estimate that **5.1%** of Beagles in the UK are carriers of the NCCD mutation. This information helps us to draw up a set of recommendations for Beagle breeders and these are shown below (**Box 1**).

Acknowledgements

We wish to thank the Kennel Club for their assistance and all participating dogs and their owners for helping us with our research.



"Madeira", NCCD clear. Photo courtesy of Sam Goldberg

Box 1: Breeding recommendations

All Beagles that are to be bred from should be DNA tested for the NCCD mutation prior to mating, regardless of sex, colour or ancestry

When planning a litter, breeders should make sure at least one of the parents is clear of the NCCD mutation (**Table 4**)

Carriers should be included in breeding programmes until the mutation frequency within the breed falls below **0.01** to avoid reducing genetic diversity unduly (see **Should we breed with carriers?** below)

Progress towards elimination of this inherited disease from the breed should be monitored by carrying out further mutation frequency checks every few years

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.

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 4** 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.

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 to 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.

Reference

Forman et al: Genome-wide mRNA sequencing of a single canine cerebellar cortical degeneration case leads to the identification of a disease associated SPTBN2 mutation. BMC Genetics 2012 13:55

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