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**The prevalence of ocular and auditory abnormalities in Merle dogs**  
**(Review of literature)**

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## **SUMMARY**

### **CLODAGH O DEA: THE PREVALANCE OF OCULAR AND AUDITORY PROBLEMS IN HOMOZYGOUS MERLE DOGS**

Merle pattern coats in dogs has been described from centuries in many breeds of domestic dog. Until recently the reason some dogs were born with this unique and beautiful pattern was a mystery. In fact it was not until 2005 that Clark et al discovered that retrotransposon insertion in SILV is responsible for merle patterning in the dog.

Even more recently it has been discovered through new research that this mysterious merle gene is not only responsible for dilution patterns but also for many auditory and ocular disorders. These disorders appear to range from rare and mild in heterozygous merles to severe and debilitating in homozygous merles.

During this thesis my aim was to discover exactly what unwanted hereditary complications are caused by the merle gene and their prevalence in the breeds associated with natural occurring merle patterns.

Furthermore I was interested in determining what precautions if any are possible to implement to avoid merle dogs suffering from these hereditary diseases associated with the gene.

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# 1. INTRODUCTION

## 1.1. THE DEFINITION OF MERLE

Merle is a term used to describe a certain coloured pattern on a dog's coat. A Merle pattern is one which creates a mottled appearance; patched so a solid colour mixed with patches of diluted version of that colour mixed throughout. These mottled patterns can be located anywhere on the dogs and can be any size and shape, edges may appear jagged or torn (figure 1).

Dogs with Merle patterned coats also frequently have dilution patterns in their eyes, so dark pigmented eyes can be blue, partially blue, or one dark and one blue. Colouring in the nose and paw pads may also have mottled dilution patterns.



Figure 1: A merle border collie

Coat colour is highly polymorphic in dogs.

In 1957 “LITTLE” described, after observing the possible phenotypes, more than 20 Loci

affecting coat colour. Until recently, only a few genes were recognised as involved in coat pigmentation. However now more and more are being discovered. One of which is the Merle Gene.

Coat colours in dogs depends on skin and hair pigment synthesis. Melanogenesis is the process by which melanocytes produce melanin, a pigment located in the skin, eyes and hair. This melanogenesis leads to a long-lasting pigmentation, which is in contrast to the pigmentation that originates from oxidation of already-existing melanin. Melanocytes manufacture two different types of melanin: Eumelanin – the black brown photo protective pigment and Phaeomelanin – the red yellow cytotoxic pigment. Many paracrine factors released mainly by surrounding keratocytes are involved in stimulating the switch between phaeomelanin and eumelanin.

Merle only affects eumelanin, which means any black, liver or blue in the coat, eyes or

nose could have the diluted merle pattern. Phaeomelanin is not affected and will appear as normal. The merle phenotype is an autosomal incomplete dominant fashion, with heterozygous dogs normally presenting a coat colour in which eumelanic regions have patches of dilute areas and homozygous merle dogs showing a more severe phenotype, these dogs are usually very pale sometimes even completely white.

## **1.2. BREEDING STANDARDS OF BREEDS CARRYING THE MERLE GENE**

For many centuries now breeders have sought to breed puppies with the Merle pattern coats. They are unusual looking and attract buyers more readily than their non-merle littermates. Some breeders can even sell merles for a higher price due to their beauty. In order to obtain the highest yield of merle puppies in a litter a breeder would be inclined to breed two merle dogs together, meaning that homozygous offspring for the merle gene would be a possibility. Due to recent studies and research that suggests the Merle gene is not only involved in causing an impressive and striking pattern on the dogs coat but is also connected to many devastating ocular and auditory hereditary diseases especially those that are homozygous for the gene, a new breed standard was released by the United Kingdom Kennel Club in 2013 with regard to breeding dogs carrying the merle gene.

In early 2012 the United Kingdom released a statement that following recommendations from the Dog Health Group, the committee had decided that it would be the last year in which they would allow the registration of puppies of any breeds which were born as a result of two merle coloured dogs being mated. They do however continue to register merles of heterozygous lineage in breeds where merle is naturally occurring. In breeds where merle is proven to not occur naturally the Kennel Club from January 2013 has not registered any dogs that are merle coloured, nor any offspring from merle coloured dogs from breeds where it does not occur naturally, even in the event of the offspring themselves not being merles.

Today there are many campaigns in the United States, for the American Kennel club to follow suit and enforce tougher regulations about breeding dogs carrying the merle gene, however to date they have not yet made any changes where merles are concerned.

### 1.3. BREEDS THAT CARRY THE MERLE GENE

While often only associated with few breeds of dog, e.g. the Collie and Catahoula Leopard Dog, Merle is in fact a distinguishing marker of many breeds including:

Border Collie,

Australian Shepherd

Shetland Sheepdog

Catahoula Leopard Dog

Cardigan Welsh Corgi

Great Dane

Chihuahua

American Pit Bull Terrier

American Staffordshire Terrier

Beauceron

Koolie

Pyrenean Shepherd

Old English Sheepdog

American Cocker Spaniel

Pomeranian

Hungarian Mudi

Norwegian Dunkerhound

It may also occur in the Dachshund but it is often referred to in literature as dappling instead of merling. In some of the breeds such as the Chihuahua and Pomeranian it is widely considered that merling is a sign of crossbreeding and so is frowned upon and unwanted.

Similarly in the American Pit Bull Terrier, historically there is no evidence of them carrying the Merle gene and is therefore considered a genetic flaw and hence the American Dog Breeding Association and United Kingdom Kennel Club do not allow registration of any American Pit Bulls exhibiting the Merle pattern.

Meanwhile in the breeding of the Border Collie, many breeders actively seek to breed

Merle coloured dogs, as buyers are more inclined to pay extra for a unique looking puppy. More importantly is the Merle gene in the Catahoula Leopard Dog, receiving its name from its spotty coat appearance. This spotty coat appearance is as a result of the Merle gene, without it the dogs would not have such dilution patterns, so in this instance breeders consider Catahoula dogs without merle colouring to be flawed. So much so that today it is extremely difficult to find a Catahoula dog not carrying the Merle gene

#### **1.4. THE DIFFERENT MERLE COLOURS**

As previously mentioned, merle pattern is a dilution. Therefore it depends on the original colour of the dog what colour merling the dog has. There are many different versions currently accepted by the Kennel Club.

##### **Blue Merle**

A mixture of smaller and larger patches covering roughly 50% of the body. Their nose pigment is black and their eyes can be blue or brown. They are able to make normal eumelanin in their coats, so their patches are black. If they didn't have the Merle gene they would be solid black. Blue Merles, as a breed standard, may also have tanned spots, registered as Blue Merle with tanned spots. Sometimes the tan points may be hard to distinguish on the dog.

##### **Red Merle**

Technically they should be called Liver Merles as the merle gene does not affect pheomelanin pigments. Red Merles have soft coloured noses, eye rims and paw pads. Some Red Merles are lighter or darker than others, there is no breed standard for how rich they must be in colour, however many people refer to those darker in colour as Chocolate Merles.

##### **Sable Merle**

Sable Merles have black noses, eye rims and paw pads. Some become so shaded as adults that the Merle pattern can be hard to distinguish.

##### **Blue Sable Merle**

Similar to a normal sable merle however the hair tips of a blue sable are grey instead of

black

### Gold Merle

Gold merles can vary in shade from a pale yellow to a deep copper colour. They may or may not have self-coloured noses, eye rims and paw pads. Like sable merles it may sometimes be hard to visually see the merle markings in a gold merle.

### Lilac Merles

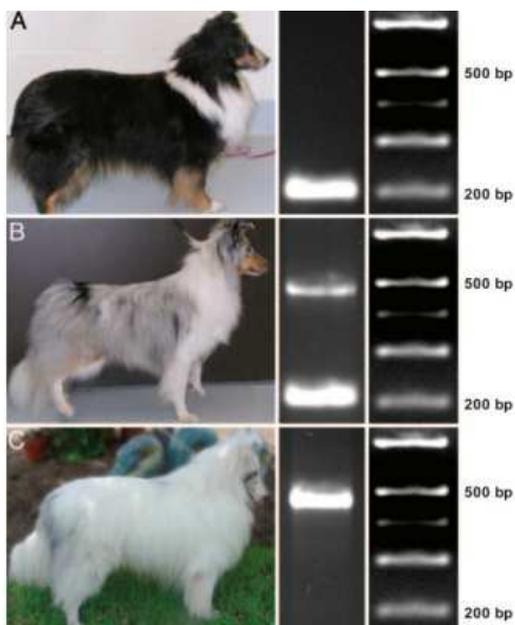
Lilac is a dilute of chocolate/red merles. These dogs can look like a pale grey colour with a purplish tinge to their coats. They have self-coloured noses, eye rims and paw pads.

Sometimes black and white dogs with ticking or roaning can be mistaken for merles. The main give away that they are not merles is that they will have a very uneven grey area, with flecks of white showing through and if they have tanned points they will be flecked as well, whereas in a merle they should be solid. Additionally their noses and eyes are not commonly affected.

## 1.5. LOCATION OF THE MERLE GENE

In 2005 L.A Clark, J.M White, L.A Rees and K.E Murphy undertook research to see if they could discover what gene was responsible for the merle patterning. Previous to this research many theories had been proposed about the merle gene

The researchers observed that many of the abnormalities associated with the merle dogs are remarkably similar to those observed in the human condition Waardenburg Syndrome. Waardenburg Syndrome is an autosomal dominant auditory-pigmentation disorder in



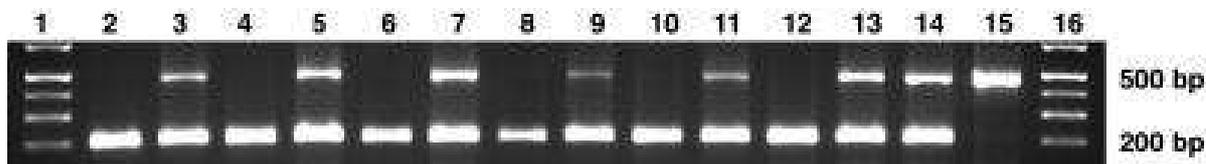
humans. There are four clinical varieties of Waardenburg Syndrome for which several genes have been implicated, e.g mutations in PAX3 causes Waardenburg types 1 and 3, mutations in SOX10 causes Waardenburg type 4 and mutations in MITF causes Waardenburg type 2, however more mutations have yet to be identified.

Using the knowledge behind the discovery of the genes causing Waardenburg Syndrome Clark et al, carried out a whole genome scan of a merle Shetland sheepdog using a multiplexed minimal screening kit to identify a chromosomal region segregating with

**Figure 2: Genetic testing of Australian Shepherds** merle. Linkage disequilibrium for merle was identified with a microsatellite marker in a region of CFA10, the region of which harbours the *SILV* gene.

The *SILV* gene is a pigment gene, responsible for the recessive trait in inbred strains of black mice in which the hair colour dilutes with age (Dunne and Thigpen, 1930). That fact and the other linked data such as significant expression of the gene almost exclusively to the skin and eye made the *SILV* gene a good candidate gene for merle.

Further studies were done on 50 more Shetland sheepdogs using the linkage analysis. The 50 dogs were analysed by gel electrophoresis for the insertion. The insert was present in the heterozygous state in 12 dogs, in the homozygous state in 2 double merles. Thirty one non-merle dogs did not harbour insertions and four non-merle dogs were heterozygous for a smaller insertion. In order to determine whether the *SILV* insertion mutation causing merle patterning in the Shetland Sheepdog was breed specific or not, merle and non-merle dogs representing six other breeds (Border Collie, Australian Shepherd, Cardigan Welsh Corgi, Dachshund, Great Dane and Collie) were analysed for the insertion. Merle dogs from all six breeds were heterozygous and one double merle Great Dane was homozygous for the insertion, proving that a mutation in the *SILV* gene is most likely the cause for the merle pattern in dogs.



**Figure 3: Mutation analysis of *SILV* and its segregation in six breeds. PCR on genomic DNA from a sable/white Collie (lane 2), blue merle Collie (lane 3), black/white Border Collie (lane 4), blue merle Border Collie (lane 5), red Australian Shepherd (lane 6), blue merle Australian Shepherd (lane 7), brindle Cardigan Welsh Corgi (lane 8), blue merle Cardigan Welsh Corgi (lane 9), black/tan Dachshund (lane 10), red dapple Dachshund (lane 11), fawn Great Dane (lane 12), blue merle Great Dane (lane 13), and harlequin Great Danes (lanes 14**

## **2. SURVEY OF LITERATURE**

### **2.1 MATERIALS AND METHODS**

In this thesis I have chosen to base my knowledge on several journals in which I have collected and are listed in the references. I utilised many journal databases for my search; such as 'pubmed', 'Science Direct' and 'CAB abstracts' to obtain several up to date publications related to my topic. I then analysed and investigated the results to which I have referenced when relevant throughout my work. I have also used several reliable webpages including Louisiana State Veterinary Education webpage, <http://www.lsu.edu/>, American Veterinary Ophthalmology website, <http://www.eyecareforanimals.com/>, The Australian Shepherd Health & Genetics Institute, <http://www.ashgi.org/>, American Dog breeders Association, [http://www.adbadog.com/p\\_home.asp](http://www.adbadog.com/p_home.asp) and United Kingdom Dog Genetics, <http://www.doggenetics.co.uk/merle.html>.

Furthermore I referenced several books "Veterinary Genetics and Animal Breeding" written by Zöldág, László, "The Merck Veterinary Manual" edited by Cynthia M. Kahn and Scott Line, "The genetics of the dog" written by A. Ruvinsky & J. Sampson, "Small animal internal medicine" by Richard W. Nelson and C Guillermo Couto and "Slatter's Fundamentals of Veterinary Ophthalmology" edited by David J. Maggs, for further information.

### **2.2. BREEDING WITH THE MERLE GENE**

In any dog, two copies of a particular gene are present, one from each parent dog. It is the same with the Merle gene which for the purpose of this example shall be called "m" and non-merle denoted as "M". If both copies are the same for having Merle, they are termed homozygous (mm) or a double merle. If one copy is merle and one is not, they are called heterozygous (Mm). One merle gene is dominant over the non-merle gene, which means

that just one copy (Mm) will produce dilution of coat and other merle characteristics. A dog that is homozygous for the non-merle gene (MM) is a normal, full coloured dog. Below is a table showing all the possible genotype combinations of dog heterozygous for the merle gene, homozygous for the merle gene and homozygous for the non-merle gene.

M= Merle, m= non-Merle

Parent 1	Parent 2	Offspring's
MM	Mm	50% full merle MM (homozygous) 50% merle Mm with carrier of solid (heterozygous)
MM	mm	100% merle dogs Mm -> carrier solid
Mm	Mm	25% merle dogs of MM (homozygous) 50% merle dogs of Mm -> carrier of solid 25% dogs with solid mm
Mm	mm	50% solid mm 50% merle Mm carriers
mm	mm	100% non-merle

Table 1 – Possible progeny

### Homozygous Merle Carriers MM

Also known as a double merle or a lethal white. A dog with one copy of the gene suffers dilution, with two copies of the gene the affect is doubled, resulting in the coat turning white.

## **Cryptic Merles**

Cryptic merle („phantom" merle) are dogs that show only very slight merle coloration and in some cases it is not visible at all. The dog can have only small patches of merle, for example, at the end of tail or ear or the merle coloration can be concealed by white markings.

These dogs carry a shorter version of the merle gene, sometimes one copy and sometimes two copies. Unlike regular merle dogs, the cryptic merle dogs apparently have no congenital problems associated with the merle gene - dogs with two copies of cryptic merle gene (Mc/Mc genotype) or dogs with one cryptic merle copy and one regular merle copy (M/Mc genotype) have no health problems. The correct description of cryptic merle is a problem when registering the dog. These dogs appear like normal coloured and are incorrectly registered as non-merle dogs.

Frequent mistakes: Excessive white markings in puppies from a tri-to-merle cross are not an indication that the puppy is a cryptic merle. The genetics of excessive white markings is completely different and have nothing to do with merle gene.

In breeding, a cryptic merle can be mated only with non-merle dogs (like dogs with regular merle allele). When crossed, the cryptic allele may expand again to regular non-shortened merle allele. When mating a cryptic merle (Mc/M) with a non-merle (M/M) you can find puppies with the following genotypes: Cryptic merle/non-merle (Mc/M), Merle/ non-merle (m/M), non-merle/non-merle (M/M).

Possible results of genetic testing:

m/m

non merle dog

M/m

merle heterozygote (visible merle pattern without health problems)

rare hidden merle heterozygote (INVISIBLE merle colour, without health problems) - high risky for breeding, if the genotype is not known

M/M

merle homozygote (visible merle pattern, severe health problems)

m/Mc

cryptic merle heterozygote with shorter gene variant (slight merle patterns can be visible, without health problems) - very risky for breeding, if the merle colour is visibly indistinguishable, the genotype is determined by genetic testing

Mc/Mc

cryptic merle homozygote with two shorter variants of merle gene (slight merle pattern can be visibly distinguished, without health problems) - very risky for breeding, if the merle colour is visibly indistinguishable, the genotype is determined by genetic testing

m/Mc

Merle / cryptic merle dog (visible merle pattern, without health problems) - high risk for breeding, if the genotype is not known.

### **2.3. PROBLEMS ASSOCIATED WITH THE GENE**

It has long since been recognised that Dalmatians who carry the piebald gene and white cats frequently have congenital problems associated with their eyes and ears, (Lurie MH, 1948). However it was not until 2006 that more in depth research was done to determine how prevalent these problems were.

#### **2.3.1 AUDITORY PROBLEMS**

Congenital deafness in dogs and cats is primarily of the hereditary sensorineural form associated with pigment dilution genes, although acquired forms of deafness are possible. This deafness results from degeneration of the cochlear blood supply at 3-4 weeks, presumably resulting from suppression of melanocytes by the merle genes. Sensory function in neonatal dogs and cats is primarily tactile, olfactory and gustatory. The visual and auditory senses, although partially functional at birth, exhibit significant postnatal development. In the dog, the eyes do not open until a puppy is between 8 and 10 days of age, the ear canals do not open until it is 12 – 13 days of age and mature system function up through the cortex is not present until it is 3 months of age or older. As a result disorders

of these systems frequently escape early detection.

Pathophysiology of deafness:

Perception of sound first requires transmission through the outer and middle ears to the cochlea for transduction by neural hair cells. Perception results from transmission of transduced auditory information from the cochlea by the eighth cranial nerve to the dorsal and ventral cochlear nuclei, the inferior colliculus, the medial geniculate nucleus of the thalamus and the primary and secondary cortical auditory areas on the temporal lobe and attention to the arriving information.

Congenital Hereditary Sensorineural Deafness:

Sensorineural hearing loss in which the root cause lies in the Vestibulocochlear nerve, the inner ear or central processing centres of the brain. The hearing loss can be mild, moderate or severe i.e. complete deafness. The majority of sensorineural hearing problems is caused by abnormalities to the hair cells in the cochlea.

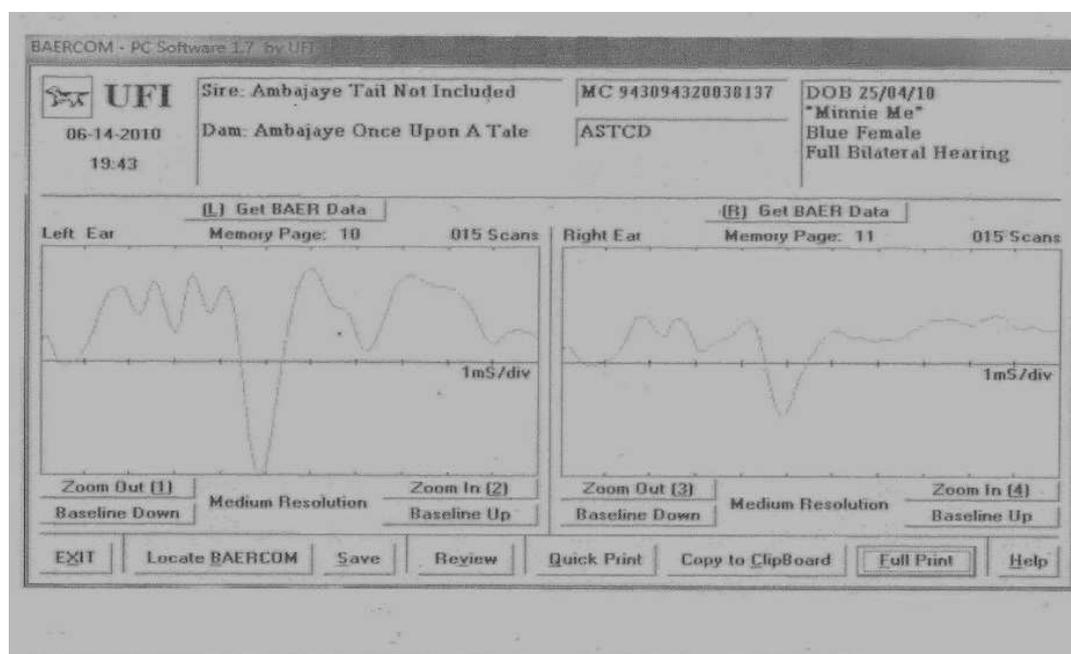
In dogs, very rarely is it associated with animals that are not of white pigmentation or carrying the piebald or Merle gene. The outer margin of the scala media is covered by a vascular bed, the stria vascularis. In pigment associated heredity deafness, the vascular bed lining degenerates. The stria is responsible for secretion of endocochlear fluid and maintenance of its high  $K^+$  concentration which is essential to sound transduction by the sensory hair cells. The exact cause for the strial degeneration is unknown but histological studies have demonstrated an absence of strial melanocytes, whose presence or postnatal development is suppressed by the merle gene.

The function of melanocytes in the stria is still unclear, but they appear to be critical to the maintenance of elevated  $K^+$  levels in the scala media and the survival of the stria. Once the degeneration has occurred the loss is permanent and there is no possibility to regenerate the cochlear neuronal tissue.

How to diagnose Deafness:

The most widely used electro diagnostic test of hearing is the brain stem auditory evoked response (BAER). The BAER detects electrical activity in the cochlea and auditory pathways in the brain, the response waveform consists of a series of peaks labelled with Roman numerals is produced by the cochlea and auditory nerve and later peaks are produced within the brain. The response from an ear that is deaf is essentially a flat line. The response is collected with a special computer through small subdermal needle electrodes: one is placed either between and behind the eyes or on the neck. A stimulus air conducted click is produced by the computer and is directed in the ear with a foam insert earphone. Each ear is tested individually.

The click stimulus used contains most of the audible frequencies of the dogs and cat, with the exception of the very highest perceived frequencies. Accordingly the BAER is a frequency nonspecific test that is more useful for detecting the presence or total absence of auditory function without quantifying hearing loss in decibels. Full maturation of the BAER occurs around day 40 of life in the dog, so must be performed after that.



In 2006 S.Platt, J. Freeman, A. di Stefani, L. Wieczorek and W. Henley conducted a study

Figure 4 - BAER test results.

to estimate the prevalence of congenital sensorineural deafness in Border Collies and investigated its association with phenotypic attributes linked to the merle gene, including coat pigmentation and iris colour. (J Vet Intern Med 2006; 20:1355-1362). Their research subjects consisted of 2,597 Border Collies who were presented to the Animal Health Trust by their owners voluntarily. All dogs included in this experiment were over the age of 6 weeks because cochlear receptor cell development is incomplete before this time and therefore an accurate BAER test would not be possible.

Before the BAER testing was carried out on any animal, some phenotypic markers were recorded including, coat colour and eye colour. In addition dogs with excess white pigmentation (which was subjectively estimated at more than 50% of the head) were recorded.

BAER tests were carried out on each dog as described above, the right ear was tested first followed by the left and the findings recorded.

Among the 2,597 tested Border Collies, 2,481 (95.5%) had normal BAER, 60 (2.3%) were unilaterally deaf and 56 (2.2%) were bilaterally deaf. Upon comparing the hearing results with the dogs phenotypic information it was discovered that significant associations with deafness were found for coat pigmentation varieties linked to the merle gene across all age categories, 145 merle dogs were included in the test, with 24 of them proving deaf (16%).



**Figure 5 - A puppy undergoing a BAER test.**

		% of Total	% of Normal Hearing	% of Deaf
Coat Colour	No.	N = 2,597	N = 2,481	N= 116
Black	1924	74.1	74.6	62.9
Red	368	14.2	14.3	12.1

Blue	129	5	5	3.5
Merle	163	6.3	5.6	10.7

**Table 2 – Results of BAER testing**

The main fault I found with this study was that the dogs were not actually genotyped for the merle allele, all work was based on the visual phenotype, consequently the distribution of heterozygous and homozygous merles could not be determined, and indeed some merles may have been accidentally placed into the non-merle category due to weak dilution patterns.

In 2009 G.M Strain took research on this matter even further by performing numerous BAER tests to determine the prevalence of deafness in dogs heterozygous or homozygous for the Merle Allele. (J Vet Intern Med 2009; 23:282-286)

His research subjects were solicited from local and national kennel clubs and breed organisations. One hundred and fifty three merle dogs became the subject of the research. Data was collected from the dogs of 10 different breeds and 1 mixed breed. Ninety-four subjects were female and fifty nine were male, ages ranged from 5 weeks to 15 years. Other data recorded included eye colour (blue versus brown), hearing status, and merle allele genotype.

All the dogs were then DNA tested to determine if they were heterozygous or homozygous for the Merle gene. Buccal cells were collected using cheek swabs and the DNA isolated using an Applied Bio systems kit. Genotyping of the dogs was accomplished by determining the presence of the short interspersed element in the SILV.

Hearing tests were carried out using the BAER method as I previously described. Owners of dogs that tested unilaterally or bilaterally deaf were then further questioned about the age of onset of hearing loss, and relevant history in an attempt to acquire causes. No affected subjects had history suggestive of non-genetic causes of deafness.

Deafness prevalence for the 153 dogs was 4.6% unilaterally deaf and 4.6% bilaterally deaf;

9.2% total was affected. For single Merles, 2.7% were unilaterally deaf and 0% was bilaterally deaf; 3.5% total was affected. For double merles, 10 % were unilaterally deaf and 15% were bilaterally deaf; 25% total was affected. A significant association between hearing status and merle genotype was observed, with double merles more likely to be affected than single merles.

Breed	N	All Merle Dogs			+/Merle			Merle/Merle		
		B	U	D	B	U	D	B	U	D
Catahoula	54	51	1	2	25	0	0	26	1	2
A. Shep.	32	29	1	2	26	1	0	3	0	2
Chihuahua	18	18	0	0	18	0	0		-	-
Collie	15	13	2	0	12	0	0	1	2	0
Sheltie	9	7	1	1	7	1	0	0	0	1
Corgi	8	7	1	0	7	0	0	0	1	0
Great Dane	6	4	1	1	4	1	0	0	0	1
B. Collie	5	5	0	0	5	0	0	-	-	-
Dachshund	4	4	0	0	4	0	0	-	-	-
Cocker Spaniel	1	0	0	1	0	0	1	-	-	-
Mix	1	1	0	0	1	0	0	-	-	-
	153	139	7	7	109	3	1	30	4	6

**Table 3 - Results of BAER test**

### **2.3.2 OCULAR PROBLEMS**

As mentioned previously the merle gene is not only responsible for a wide variety of beautiful coat colours, it is also responsible for variations of the iris or coloured part of the eye. A combination of colours may be found in one or both eyes. Colours expressed may range from a pale, light blue to greenish or amber. Unfortunately as with the dilution of hairs in the cochlea, the same gene responsible for these desirable eye colours may also lead to developmental eye defects.

In relation to ocular effects, the merle gene's only manifestation may be expression of a normal, healthy blue iris; this blue appearance may also be as an inclusion or as a partial segment of an otherwise brown eye (heterochromia iridis). A blue iris does not always indicate the presence of the merle gene as the piebald gene found predominantly in the Dalmatian also causes such changes. While there is no adverse consequence of merle dog having a blue iris alone, there are other effects of the merle gene which may result in complete blindness.

While there have not been the same extent of studies done on Merle sight problems in comparison to hearing problems, it is still accepted by most that as in the case of hearing anomalies, the most severe eye abnormalities occur in homozygous merles with an excessive white hair coat involving the head region. There are several ocular problems associated with the Merle gene, the abnormalities affect either the front or back of the eye or a combination of both.

Microphthalmia:

A defect early in development results in smaller than normal eyes. Dogs affected with this condition have prominent third eyelids and small eyes which appear recessed in the eye socket (enophthalmos). In general, this condition is evident as soon as a pup's eyes are opened. Where changes are mild there is usually no visual impairment, however in moderate cases where the eyeball fills only about half of the opening, more than 50% of these pup's will be visually impaired. There is no treatment for microphthalmia itself as it is a structural defect. Complications often arise due to microphthalmia, e.g Glaucoma, which are treated as necessary.



**Figure 6 - Persistent pupillary membrane, coloboma and contralateral microphthalmia in a merle Australian Shepherd.**

#### Coloboma:

Iris coloboma occurs when part of the iris fails to develop. Colobomas may be only small notches out of the inner edge of the iris, pie slices, or massive holes – occasionally so large it appears the dog has no iris at all. This condition is present at birth and in most cases the effect on vision is minimal. However a large coloboma can force a dog to squint in bright light because the iris is incapable of contracting properly to reduce the amount of light entering the eye. This can cause minor discomfort as well as temporarily reducing the range of vision, which could impact performance or work. Choroidal colobomas may also occur, in this condition the vascular layer at the back of the eye develops incompletely.

Posterior segment anomalies may also affect the optic nerve. The function of the optic nerve is to transmit information from the eye to the brain to enable interpretation of visualisation. A defect at this level may cause complete blindness. The mode of inheritance for colobomas is unknown. Almost all colobomas are seen in merle dogs, however they will occasionally be found in non-merles. The reason for the high association with merles is not known. It is very possible that non-merles with normal irises might carry the genes.

#### Persistent Pupillary Membrane:

The pupillary membrane is a foetal structure that covers the pupil prior to birth. It is supposed to resolve shortly after birth. Sometimes part or all of it will persist. Most persistent pupillary membranes do not cause significant visual deficit, however some, in particular those that attach to the lens or the cornea, can cause blinding opacities. Persistent pupillary membranes which have not resolved by the time a dog is a year old is considered hereditary. Though the mode of inheritance is unknown it is very common in merles, more so than non-merles.

#### Retinal Pigment Epithelium:

Prenatal studies of merle Australian Shepherds have demonstrated a primary defect in the retinal pigment epithelium, resulting in hypoplasia of the adjacent choroid and sclera (Cook et al, 1991). It is likely that the subalbinism is associated with abnormal retinal pigment epithelium that fails to induce the overlying neural crest.

#### Corectopia:

Also known as eccentric pupils is another ocular abnormality frequently seen in double merle dogs. It is the displacement of the eye from its normal central position. It can occur in one or both eyes. Corectopia, when uncomplicated, does not interfere with the dogs' vision.

#### Lens Luxation:

The lens can either become loosened (subluxated) or completely detached (luxated) from the zonules that hold the lens in place. When the lens completely tears free of its zonular attachments and falls forward into the anterior chamber, it is called an anterior luxation. It

is also possible for the lens to luxate posteriorly into the vitreous body. A luxated lens severely impedes sight if not rendering the dog totally blind.

#### Cataracts:

Cataracts are one of the most common dog eye diseases. Lens opacities can be caused by a number of things, but hereditary cataracts will always be bilateral, though one eye may develop them six months to a year before the other. Some remain small but others will progress until the dog has lost all functional vision. Most cataracts seen in merles are posterior polar, meaning they start in the middle of the back side of the lens. Age of onset for hereditary cataracts varies widely from as early as 18 months into old age.



**Figure 7 - Cataracts in a Merle dog**

#### Retinal Dysplasia:

Can involve just one or both retinas. It caused by an abnormal development of the retina, whereby two primitive layers of the retina fail to form together properly, resulting in retina folds. These folds can be focal or multifocal, which appear as streaks or dots in the central retina. Alternatively they can be geographical folds, which appear as irregular or horseshoe-shaped areas of mixed hyper or hyporeflectivity in the central retina. Retinal detachment occurs with complete retinal dysplasia, and is accompanied by blindness in that eye

Lack of tapetum:

In some dogs with merle colouring, the tapetum (reflective layer at the back of the eye) is missing. These dogs tend to suffer from poorer night vision in comparison to a dog with a tapetum, but there is no obvious functional abnormality with these dogs.

Unfortunately for many homozygous merle dogs they do not suffer from just one of the aforementioned eye conditions, instead usually they suffer from many. This anomaly is commonly referred to as Merle Ocular Dysgenesis.

Merle Ocular dysgenesis in Border Collies, Australian Shepherds and Shetland Sheepdogs is often misdiagnosed as Collie Eye Anomaly. Collie Eye Anomaly is also a congenital bilateral eye disease of dogs, which affects the retina, choroid and sclera. It is caused by a simple autosomal recessive gene and is not connected to the merle gene, therefore can be seen in any colour dog and not just in merles.

	Merle Ocular Dysgenesis	Collie Eye Anomaly
Coat Colour	Homozygous Merle	No correlation
Microphthalmia	Frequent	Rare
Choroidal Hypoplasia	Extensive sclera and retinal	Common but localised
Optic Nerve Coloboma	Rare	Frequent
Cataracts	Frequent	Rare
Iris Coloboma	Frequent	Rare

**Table 4 - Comparative feature of Merle Ocular Dysgenesis and Collie Eye Anomaly**

### **3. DISCUSSION AND CONCLUSIONS**

For many decades now homozygous merle dogs have been suffering from hereditary ailments due to our lack of knowledge and assertiveness in controlled breeding measures. It was only during my research for this thesis that I realised just how many and to what extent homozygous merles really suffered, with up to 25% of them being effected by disease (G.M Strain 2009). I have researched why this is the case and how we can improve the current situation.

#### **Breeding in the past**

The Kennel Club has a breeding standard outlined for every breed of dog which it recognises. A breeding standard is a set of guidelines covering specific externally observable qualities such as temperament, appearance and movement. The Kennel Club states that the “form and function and fitness for purpose” are key features of the breeding standard, however until 2012 in the UK and to this day in the USA, they have been allowing dogs naturally carrying the merle gene to be bred without restriction. This means that many homozygous merles were and are being born with ocular and auditory deformities and not fit for purpose as the Kennel Club states.

The Associate Parliamentary Group for Animal Welfare in 2010 undertook a large scale enquiry into the health and welfare issues surrounding the breeding of pedigree dogs. They released a report in November of that year that discussed the serious problem with the health and welfare of many pedigree dogs and how they can be caused by worrying breeding practices. The report made many recommendations, one of which proved excellent for the merle dogs, it stated that “Breed Standards should be based less on visual aesthetics and more on whether dogs are healthy and fit for purpose.”

Catahoula Leopard dogs and Border Collies are two of the most commonly found merle dogs and are both working and herding dogs. If born blind and deaf these dogs while a beautiful colour are certainly not fit for their purpose.

## Breeding goals and standards today

In early 2012 after the Associate Parliamentary Group for Animal Welfares report was released the United Kingdom Kennel announced many changes in their breeding standards and goals for dogs. One of these changes was for dogs who naturally carry the merle gene. Enforced from January 2013, no two dogs carrying the merle gene can be bred together and therefore no homozygous puppies born can be registered with the Kennel Club. The United Kingdom Kennel Club General Committee also banned registration and progeny produced by a merle coloured Chihuahua. The Kennel Club stated that they seek to eliminate the merle gene from the Chihuahua completely as it was not naturally occurring and instead introduced to the breed. Essentially by barring the breeding of any Chihuahua with a merle pattern they should be eliminated soon.



Figure 8 - An example of a properly bred Merle dog

Unfortunately to date the American Kennel Club has not made any changes with regard to regulating merle breeding. Chihuahua's carrying the Merle gene, even though it is not a naturally occurrence, is permitted and they are allowed to be registered and shown in breed shows. Homozygous merles of all recognised breeds are also allowed to be registered and bred from with other merles with no regard to the health problems this may possess.

An example of such breeding is evident in one of the most prestigious Collie breeding centres in America, Wyndlair Collies. On their webpage they advertise Wyndlair Avalanche for stud use, they describe him as follows "This exquisite homozygous blue merle is everything we hoped for from such beautiful parents. Aiden (his pet name) possesses beautiful high cleanness and lightness of head with a pretty profile, huge outline, strong rear and breath taking presence. From his birth, we knew that Aiden was destined to be truly special. As his puppies have been completed their Championships in amazing style, our belief in him has been affirmed". What the breeders conveniently fail to mention is that Aiden cannot compete himself in these championships as he cannot see his way around the ring because he is blind due to his homozygous breeding. In my opinion, this is a perfect example of a lack of ethics in pursuit of aesthetics. It is fundamentally wrong to intentionally inflict disease upon dogs for the sake of producing a few more blue merle puppies each litter.

### **The future of the Merle**

In my opinion the future of the merle patterned dog is a bright one. In the UK great strides have been made to ensure that in the future no homozygous merles will be bred and therefore suffer from the debilitating ocular and auditory diseases associated with them.

However further work could be done to ensure the health of the merle dogs.

As discussed previously Cryptic Merles can occasionally be born, with very little physical features of being a merle. Therefore they could easily be dismissed as a non-merle and bred to a merle, producing homozygous progeny. All dogs who have the potential to carry the merle gene, i.e. have a parent who is a merle, should be subjected to a DNA study to ensure that they are not a cryptic merle. There is no such regulation about this yet to date.

Also discussed previously was the BAER testing, to determine the hearing status of dogs, it

should be implemented that all merle dogs that people desire to breed from have a BAER test after they are six weeks of age. Any dogs whose results are not satisfactory should be unable to be bred from and disqualified instantly from the future gene pool.

Similarly to avoid the continuing spread of ocular deformities in merle dogs, all merles being bred from should have to undergo a CERF examination. CERF, or the Canine Eye Registration Foundation is an organization that tracks heritable eye diseases in dogs with the intention of monitoring breeding dogs, and with the greater hope of ultimately eliminating these conditions. A CERF exam is valid for one year. Ideally, dogs should be certified every year by a veterinary ophthalmologist to ensure that conditions that may be progressive or develop later in life have not appeared. These exams do not guarantee that the dog is not a carrier of genetic ocular disease; rather, a passing test proves that at that time no genetic ocular disease was diagnosed. However it is a step in the right direction in preventing the spread of heredity diseases.

Hopefully in the very near future, the American Kennel Club will follow in the footsteps of the United Kingdom and tighten regulations surrounding the breeding of merle dogs to ensure a healthier, happier life for merle dogs worldwide.

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