Prevalence of Deafness in Dogs Heterozygous or Homozygous for the Merle Allele


Background: Deafness in dogs is frequently associated with the pigment genes piebald and merle. Little is known about the prevalence of deafness in dogs carrying the merle allele.

Objective: To determine the prevalence of deafness in dogs heterozygous and homozygous for the merle allele of the mouse Silver pigment locus homolog (SILV) gene.

Animals: One hundred and fifty-three privately owned merle dogs of different breeds and both sexes.

Methods: Hearing was tested by brainstem auditory-evoked response and classified as bilaterally hearing, unilaterally deaf, or bilaterally deaf. DNA from buccal cells was genotyped as either heterozygous or homozygous for the merle allele. Deafness association tests among merle genotype, eye color, and sex were performed by the χ² test.

Results: Deafness prevalence in merles overall was 4.6% unilaterally deaf and 4.6% bilaterally deaf. There was a significant association between hearing status and heterozygous versus homozygous merle genotype. For single merles (Mm), 2.7% were unilaterally deaf and 0.9% were bilaterally deaf. For double merles (MM), 10% were unilaterally deaf and 15% were bilaterally deaf. There was no significant association with eye color or sex.

Conclusions: Deafness prevalence in merles was greater than that in some dog breeds homozygous for the piebald gene, such as the English Cocker Spaniel, but comparable to, or lower than, that in the Dalmatian and white Bull Terrier. Dogs homozygous for the merle allele were significantly more likely to be deaf than heterozygotes.

Key words: Allele; Genotype; Piebald; SILV.

Congenital hereditary deafness in most dog breeds is associated with 1 of 2 classical pigmentation genes responsible for white or light skin and fur coloration: piebald and merle.¹ The pigment locus S has 3 recessive alleles: Irish spotting, piebald, and extreme piebald; dogs with the dominant allele have solid color. Dogs homozygous for 1 of the recessive alleles have white coloration. Dogs with any of the recessive alleles may have congenital hereditary deafness. The prevalence varies by breed, and can be as high as 30% (unilateral and bilateral deafness combined).²

A dominant allele (M) of the pigment locus mouse Silver pigment locus homolog (SILV) produces a pattern of random patches of diluted pigmentation alternating dark versus light over an underlying uniform coloration. This coat color pattern is called merle and is known as dapple in some breeds. Prevalence of deafness in merle breeds is not well documented. Evidence suggests that deafness is inherited in a non-Mendelian manner in piebald breeds,³ but similar studies have not been performed in merle breeds. The merle pattern is seen in the Collie, Australian Shepherd, Shetland Sheepdog, Catahoula Leopard Dog, Cardigan Welsh Corgi, Dachshund, and Great Dane breeds, and less commonly in the Chihuahua, American Pit Bull Terrier, American Staffordshire Terrier, Beauceron, Border Collie, Koolie, Poodle, Pyrenean Shepherd, Old English Sheepdog, American Cocker Spaniel, Pomeranian, Hungarian Mudi, Norwegian Dunker Hound, and others. In addition to deafness, dogs with merle, particularly double merles (MM), may have ophthalmic disorders.

Previous work showed that a retrotransposon in the SILV gene is causative for the merle pattern.⁵ However, it is not yet known how these genes affect hearing.

Relatively few studies of the merle phenotype have been published. Among these is a study from a research colony of Dachshunds (Tekels in German) maintained in Hanover, Germany involving auditory function,⁶ with measurements taken from 38 dogs. This study reported a deafness prevalence (unilateral or bilateral) of 54.6% in double merles and 36.8% in single merles. The findings in this study were limited to a small established population of 1 breed and have, unfortunately, been extrapolated to all breeds having the merle allele. A more recent study of Border Collies in the United Kingdom⁷ reported a deafness prevalence of 2.8% (N = 64; 2.3% unilateral, 0.5% bilateral) in 2,303 puppies. A significant correlation between the merle coat pigmentation pattern and deafness was observed. However, dogs were not genotyped for the merle allele, and consequently the distribution of heterozygous and homozygous merles could not be determined.

The research described in the present report and records of many breeders suggest that the deleterious effects of the merle allele may have been overstated in some publications. In the present study, we used a population of both related and unrelated merle-genotyped dogs to assess the prevalence of deafness in heterozygous and homozygous merles, and describe differences among breeds.
**Materials and Methods**

**Animals**

Research subjects were solicited from local and national kennel clubs and breed organizations by electronic methods; correspondence with these organizations emphasized a desire to study merle dogs both with normal hearing and known or suspected deafness to decrease any possible bias for either hearing state. Owners were told that the study was an attempt to quantify deafness in merle dogs, and that the results would be confidential. Breeders of breeds in which the merle allele is under scrutiny for possible exclusion from the breed standard likely were motivated to enroll hearing dogs to protect the permissibility of the merle pattern. It is unknown if such bias led to subject participation that was not random.

The majority of the subjects were from Louisiana or Texas. This fact may have led to geographical distribution bias, but it is unclear what factors might have led to regional differences. One hundred and sixty-one dogs participated in the study; of these, 8 proved to be nonmerle, resulting in 153 merle subjects. Most were purebreds, but 5 dogs were mixed breed. All but 1 mixed breed dog phenotypically strongly resembled a known breed (2 Australian Shepherds, 1 Dachshund, and 1 Great Dane), and these dogs were included as members of the respective breed for analysis. The study design was approved by the Louisiana State University Clinical Study Protocol Review Committee (#06-37) and data were collected with owner informed consent.

Data were collected from dogs of 10 breeds and from 1 indeterminate mixed breed dog. The largest number of subjects came from the Catahoula (54), Australian Shepherd (32), Chihuahua (18), and Collie (15) breeds (Table 1). Ninety-four subjects were female and 59 were male. Ages ranged from 5 weeks to 15 years (mean, 2.9 years). Data included breed, sex, eye color (blue versus brown), hearing status, and merle allele genotype.

**Hearing Testing**

Hearing was assessed using brainstem auditory-evoked response (BAER) recordings. Subdermal needle electrodes were placed over the vertex and adjacent to the auditory canal, with a ground electrode over the neck. Broadband rarefaction click stimuli (0.1 ms duration, 11.33/μs, 95 dB nHL) were applied by means of insert earphones, 1 ear at a time, without contralateral masking noise or chemical restraint. Between 500 and 1,000 responses of 10 ms duration were averaged. Dogs were classified as bilaterally hearing if the typical multiphasic BAER responses were present in both ears, as unilaterally deaf if only 1 ear had a response, and as bilaterally deaf if neither ear responded. No attempt was made to determine hearing thresholds or assess partial hearing loss, because the clinical manifestation of pigment-associated deafness is either total deafness or normal hearing in a given ear. All subjects were BAER tested by 1 of the authors (GMS) except for 5 subjects that were tested at other established testing sites (Texas A&M University, Cornell University, North Carolina State University, and 1 private veterinary practice); copies of graphical results for these dogs were available, enabling confirmation of the hearing status (BAER pattern present or absent). Other equipment was used at these sites, but test stimulation and recording protocols were similar based on parameter settings printed on test results.

Owners of dogs that tested as unilaterally or bilaterally deaf were questioned about the age of onset of hearing loss, if known, and relevant history in an attempt to identify acquired causes, such as presbycusis, otitis, sound trauma, or ototoxicity. No affected subjects had a history suggestive of nongenetic causes of deafness.

**Merle Genotyping**

Buccal cells were collected using cheek swabs, and DNA was isolated using commercial kits. Genotyping of dogs as nonmerle (+/+), heterozygous merle (+/merle), or homozygous (double) merle (merle/merle) was accomplished by determining the presence of the short interspersed element (SINE) insertion in \( \text{SILV} \). \(^5\) PCR amplification of exon 11 of the \( \text{SILV} \) gene was achieved with exon 11 forward (CAG TTT CTC CTT TAT TCT CCC A) and exon 11 reverse (CCT CGG CAA ATC ACA GCA) primers. PCR was carried out as described previously.

**Statistical Analysis**

Deafness association analyses were performed using the \( \chi^2 \) test by a commercial software program. Fisher’s exact probability test was used when cell counts in \( \chi^2 \) contingency table cells were too small. Associations were considered significant for \( P < .05 \). Individual data points in these analyses cannot be considered strictly independent because various familial relationships were present (eg, littermate, father-daughter). However, because pedigree relationships were not known for most subjects, pedigree-based analyses could not be performed and the data were by necessity treated as being independent. Prevalence comparisons across breeds could not be performed because of small subject numbers.

**Table 1. Deafness prevalence in merle dogs by breed and by merle genotype.**

<table>
<thead>
<tr>
<th>Breed</th>
<th>All Merle Dogs</th>
<th>+/Merle</th>
<th>Merle/Merle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>B</td>
<td>U</td>
</tr>
<tr>
<td>Catahoula</td>
<td>54</td>
<td>51</td>
<td>1</td>
</tr>
<tr>
<td>Australian Shepherd</td>
<td>32</td>
<td>29</td>
<td>1</td>
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<td>Chihuahua</td>
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<td>18</td>
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<tr>
<td>Collie</td>
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<td>2</td>
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<tr>
<td>Shetland Sheepdog</td>
<td>9</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Cardigan Welsh Corgi</td>
<td>8</td>
<td>7</td>
<td>1</td>
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<tr>
<td>Great Dane</td>
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<td>1</td>
</tr>
<tr>
<td>Border Collie</td>
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<td>5</td>
<td>0</td>
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<tr>
<td>Dachshund</td>
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<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Cocker Spaniel</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mix</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

| Total            | 153| 139(90.8%) | 7(4.6%) | 7(4.6%) | 109(96.5%) | 3(2.7%) | 1(0.9%) | 30(75.0%) | 4(10.0%) | 6(15.0%) |

\( ^a \) B, bilaterally hearing; U, unilaterally deaf; D, bilaterally deaf; +/merle, heterozygous merle; merle/merle, homozygous merle.
Results

Deafness prevalence for the 153 dogs was 4.6% unilaterally deaf and 4.6% bilaterally deaf; 9.2% total were affected (Table 1). For single merles, 2.7% were unilaterally deaf and 0.9% were bilaterally deaf; 3.5% total were affected. For double merles, 10% were unilaterally deaf and 15% were bilaterally deaf; 25% total were affected. A significant association between hearing status and merle genotype (+/merle, merle/merle) was observed ($\chi^2 P = .0001$, Fisher's exact test $P < .0001$), with double merles more likely to be deaf than single merles.

Eye color data was available for 144 of the 153 dogs; of these 86 (59.7%) had 1 or 2 blue eyes. Among bilaterally hearing dogs, 79 of 136 dogs (58.1%) had 1 or 2 blue eyes; among unilaterally deaf dogs, 3 of 4 dogs had 1 or 2 blue eyes; and among bilaterally deaf dogs, 4 of 4 dogs had 1 or 2 blue eyes. There was no significant association between hearing status and the eye color pattern of 2 brown eyes, 1 blue eye, or 2 blue eyes ($\chi^2 P = .128$, Fisher's exact test $P = .152$). There also was no significant difference in deafness prevalence between sexes ($\chi^2 P = .838$, Fisher's exact test $P = .915$).

Subject numbers within breeds were too small for confidence that statistical comparisons could be meaningful with the resultant sparsely populated and unbalanced $\chi^2$ table. In the 2 breeds with the largest number of subjects (Catahoula and Australian Shepherd), only 5.9% of Catahoulas were affected (533/54), whereas 9.4% of Australian Shepherds were affected (33/32). Of the double merles, only 3 of 29 Catahoulas were affected (10.3%), whereas 2 of 3 Australian Shepherds were affected (66.7%).

Discussion

Deafness prevalence rates (unilateral and bilateral) in dogs with the merle allele were 9.2% overall, or 3.5% in single merles and 25% in double merles. These overall values are compatible with, or below, those reported in dogs homozygous for the various alleles of the piebald gene. A prevalence in Dalmatians in the United States is 22% unilaterally deaf and 8% bilaterally deaf, or 30% affected, and 20% in white Bull Terriers, whereas in the English Setter 12% are affected and in the English Cocker Spaniel only 6% are affected. The risk for deafness in single merles seems to be no higher than that in some piebald breeds such as the Dalmatians and the white Bull Terrier, and even in double merles the risk is not as high as indicated in some publications. However, the 15% bilateral deafness prevalence rate in double merles is higher than in piebald breeds; this may be a reflection of the small sample size or other unrecognized factors. Also, the impact of the merle allele on auditory function may vary with breed (as also is the case for piebald breeds) as indicated by the differences between Catahoulas and Australian Shepherds. Based on unpublished observations (GMS), the collie-type breeds (Collie, Sheltie, and Border Collie) appear to be more affected by deafness than some other breeds such as the Catahoulas. However, larger numbers of BAER-tested and genotyped subjects will be required to determine whether or not significant breed differences exist.

From the total non-Catahoula $MM$ population of the present study, 7 of 11 were affected (63.6%), suggesting a possible difference between the Catahoula and other merle breeds for merle effects on hearing. This finding is not surprising because most double-merle Catahoulas are heavily pigmented compared with double merles of other breeds. One of the 2 bilaterally deaf double-merle Catahoulas was nearly all white, whereas the other was a normal red merle. None of the other single- or double-merle Catahoulas had unusual amounts of white pigmentation.

A biological basis for breed differences in deafness prevalence may come from molecular studies reporting breed differences in the mutations responsible for the pigmentation pattern. The genetic basis for the piebald allele has been suggested to be 2 candidate mutations in a 3.5-kb region upstream from the $M$ promoter of the microphthalmia-associated transcription factor ($MITF$) gene: a SINE insertion present in all tested extreme piebald ($s^0$) and piebald ($s^2$) breeds, but absent in all Irish spotting ($s^i$) and solid ($S$) dogs, and a unique length polymorphism (shortening of the promoter region) in solid dogs. Dalmatians, which are $s^0$, carry the SINE insertion but a short allele at the polymorphism site, suggesting a unique mutation. Dalmatians have the highest prevalence of deafness of all piebald breeds studied, possibly related to this unique $MITF$ mutation. However, the sequence of the $SILV$ SINE insertion in merle breeds was identical in the 7 breeds examined except for a single point mutation in 2 breeds; thus, polymorphisms in pigment gene alleles may not explain breed differences in deafness prevalence if the $SILV$ SINE insertion is the determining factor.

Platt et al. BAER tested 2,597 Border Collies in the United Kingdom; 2,303 of these were puppies, and deafness prevalence rates of 2.3% unilateral and 0.5% bilateral deafness were determined. This study included both merle and nonmerle dogs. When the additional 294 adults were included, the total prevalence increased to 2.3 and 2.2%, probably reflecting a selection bias. These values are very similar to the prevalence rates for single merles obtained in the present study (2.7 and 2.3%); the authors found a significant association between deafness and dogs with merle or excess white coat pigmentation. Because genotyping was not performed, it is not known whether the dogs with excess white pigmentation were double merles or whether the pattern reflected the influence of a co-existing piebald gene. All Border Collies are thought to be homozygous for any of the 3 piebald recessive alleles, and it is unknown what interaction may occur between merle and piebald.

No statistical association was observed between the presence of blue eyes and deafness in merle dogs of this study, which was surprising considering the well-documented association in dog breeds carrying heterozygous recessive piebald alleles. Both the piebald and merle genes produce blue eyes by suppressing melanocytes in the iris and deafness by suppressing melanocytes in the stria vascularis of the cochlea; thus, the association in piebald
breeds has a logical basis. A similar association would have been expected in merle breeds. The lack of association in merles might be explained by small subject numbers: only 8 of the 14 hearing-affected dogs of this study had identified eye color, and 7 of them had blue eyes.

The merle color pattern results from variable dilution of the underlying coat color. In many breeds, the merles can be split into what are known as blue merles and red merles based on the type of melanin produced by melanocytes. The melanocortin receptor 1 gene (*MC1R*) causes production of eumelanin (black, brown, or blue) when its dominant allele is present, and phaeomelanin (red, yellow, or cream) when its recessive allele is present. However, other genes modify *MC1R* effects: the tyrosinase-related protein 1 gene (*TYRP1*) determines whether eumelanin is black or brown, and the agouti-signal protein gene (*ASIP*) creates colors ranging from cream, to yellow, to red, to sable, to black-and-tan, brown-and-tan or even black. Some breeds carrying the appropriate merle alleles, such as the Chihuahua, manifest their merle patterns as fawn, sable, chocolate, and others in addition to red and blue, and it is not always obvious which *MC1R* allele is present. Consequently, there was no straightforward way to compare merle background colors in the dogs in this study to examine whether there were deafness prevalence differences among different merle colors. However, such color differences would not be expected based on studies of breeds with the piebald gene, in which no significant color differences were seen. Reetz et al reported hearing results for 38 Dachshunds: 8 nonmerles, 19 single merles, and 11 double merles. They reported hearing loss (slight to total, unilateral, or bilateral) in 54.6% of the double merles, and in 36.8% of the single merles (43.3% total merles affected), but in none of the nonmerles. Hearing was tested by determining the BAER threshold to click stimuli under sedation. Any threshold > 20 dB was designated as abnormal, not because it is an accepted standard, but apparently because 1 of the nonmerle dogs was determined to have a 20 dB hearing threshold. Only 1 dog, a double-merle male, was totally deaf in both ears (threshold > 90 dB) and none of the dogs was totally deaf in only 1 ear. If deafness is defined as the absence of a response to the loudest stimuli presented, true bilateral deafness occurred in 9.1% (1/11) of the double merles and 0% of the single merles, not the 54.6 and 36.8% reported by Reetz et al. The difference in results is explained by the inclusion by Reetz of dogs with partial hearing loss.

The pigment-associated deafness associated with the piebald and merle patterns typically presents as total deafness in 1 or both ears, based on histological studies and 1 investigator’s (GMS) experience BAER testing several thousand dogs; thus, the partial hearing losses reported by Reetz seem unlikely to be likely genetic or associated with the merle gene. Instead, they most likely reflect a combination of limited aural hygiene and otitis media, both of which cause conductive hearing loss, and otitis interna and noise-induced hearing trauma, both of which cause sensorineural hearing loss. The noise level in institutional kennels is high, based on the authors’ experience, and exposure to high noise levels produces cumulative hearing loss with time. Dogs in large kennels often do not receive regular ear cleaning, leading to build up of excessive cerumen, and develop infections, because of humid environmental conditions, use of hoses to clean facilities, and the common use of floppy-eared breeds in kennel colonies. Both excessive cerumen and otitis externa or media muffle sounds reaching the inner ear. Interestingly, of the 15 "hearing-impaired" ears with thresholds between 25 and 50 dB in the Reetz study, only 3 were in males. Differences in kennel housing for females may have exposed them to greater noise levels in the whelping kennels.

Dogs having the merle genotype but not expressing the merle phenotype are known as cryptic merles (represented here by M). The SINE insertion that causes merle patterning contains a polyA tail. To have the merle phenotype, the polyA tail must be sufficiently long (90–100 adenine repeats). If the tail is truncated to < 65 adenine repeats, the dog does not express the merle phenotype, even though it carries the SINE insertion. Two Australian Shepherds genotyped in this study illustrate this situation. A tested sire was a single merle whereas the dog’s tested daughter was a double merle. The daughter’s dam (not genotype tested) was described by the owner as a black tricolor without any merle pattern. When bred, the daughter produced both merle and tricolor phenotypes. Because the daughter was a double merle but produced phenotypically nonmerle pups, 1 of her merle alleles must contain a truncated polyA tail. The dam must be a cryptic merle to explain a daughter that is a double merle, and the tricolor offspring of the daughter must also be cryptic merles because they can only inherit a normal merle allele or a cryptic merle allele from her. A heterozygous cryptic merle (Mm), when bred to a heterozygous normal merle (Mm), can produce heterozygous cryptic merles (Mmm), heterozygous normal merles (Mmm), nonmerles (mm), and homozygous merles (MM), the last displaying the phenotype of a heterozygous merle. The merle genotyping in the present study did not assess polyA tail length. However, no heterozygous cryptic merles were included in the present study because all dogs exhibited the merle phenotype; some of the genotyped double merles could have carried 1 cryptic merle allele, including the daughter described above. It is not known to what extent cryptic merles are present in merle breed populations.

The 18 Chihuahuas in this study all were single merles, and none was deaf in either ear. There is debate among US Chihuahua breeders about the acceptability of merle in the breed, and efforts have been made to remove merle as an allowed pigmentation pattern because of a perceived risk of deafness and visual defects in merle dogs. Similar efforts to exclude double merles have occurred within the Dachshund breed in the United States; none of the 4 Dachshunds in this study, all single merles, had a deaf ear.

Because merle and piebald are caused by different genes, the coat patterns are not mutually exclusive. Having only genotyped for the merle mutation, we cannot eliminate the possibility that some dogs also carried piebald mutations (a DNA test for piebald is not available).
Consequently, the deafness in this study cannot be positively attributed to the SILV mutation. At least 3 dogs in this study appeared to carry both merle and piebald. They were from breeds known to carry both genes, and had pigmentation patterns of both the light versus dark of merle and large areas of the white of piebald. All were single merles, and 1 was unilaterally deaf whereas the other 2 had normal hearing. The MITF piebald gene (on chromosome 20) is a transcriptional regulator of the SILV merle gene (on chromosome 10), but no studies to understand the impact of having both piebald and merle pigmentation patterns have been published. The SILV gene has been eliminated as a cause of deafness in the Dalmatian breed.

Deafness prevalence in dogs with the merle allele was higher in double merles than in single merles, but the relative risk of deafness was less than that in Dalmatians and white Bull Terriers and greater than that in other dog breeds with the recessive piebald alleles for which data is available.

Footnotes

\footnote{a} Sierra or Sierra Wave electrodiagnostic systems, Cadwell Laboratories, Kennewick, WA
\footnote{b} Applied Biosystems, Foster City, CA
\footnote{c} SAS for Windows, version 9.1.3, SAS Institute Inc, Cary, NC

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References