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ORIGINAL INVESTIGATION

The *PMEL* gene and merle (dapple) in the dachshund: cryptic, hidden, and mosaic variants demonstrate the need for genetic testing prior to breeding

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Abstract

One of the most unique coat color patterns in the domestic dog is merle (also known as dapple in the dachshund breed), characterized by patches of normal pigmentation surrounded by diluted eumelanin pigment. In dogs, this striking variegated pattern is caused by an insertion of a SINE element into the *PMEL* gene. Differences in the length of the SINE insertion [due to a variable-length poly(A)-tail] has been associated with variation in the merle coat color and patterning. We previously performed a systematic evaluation of merle in 175 Australian shepherds and related breeds and correlated the length of the merle insertion variants with four broad phenotypic clusters designated as "cryptic", "atypical", "classic", and "harlequin" merle. In this study, we evaluated the SINE insertions in 140 dachshunds and identified the same major merle phenotypic clusters with only slight variation between breeds. Specifically, we identified numerous cases of true "hidden" merle in dachshunds with light/red (pheomelanin) coats with little to no black/brown pigment (eumelanin) and thus minimal or no observable merle phenotype. In addition, we identified somatic and gonadal mosaicism, with one dog having a large insertion in the harlequin size range of M281 that had no merle phenotype and unintentionally produced a double merle puppy with anophthalmia. The frequent identification of cryptic, hidden, and mosaic merle variants, which can be undetectable by phenotypic inspection, should be of particular concern to breeders and illustrates the critical need for genetic testing for merle prior to breeding to avoid producing dogs with serious health problems.

Introduction

Merle is a common coat pattern in domestic dogs that consists of patches of diluted pigment mingled with areas of normal pigmentation. The merle trait is inherited in an incomplete autosomal dominant fashion. Dogs that inherit a single copy of the merle mutation (m/M) can have a variety of merle phenotypes, and dogs that inherit two copies of the mutation (M/M) can have large areas of depigmentation resulting in white fur and significant auditory (Platt et al. 2006) and ophthalmologic (Gelatt et al. 1981) anomalies. The molecular basis of merle was originally described by Clark et al. who identified a single copy of a 253 bp SINE insertion in the premelanosome protein (*PMEL*) gene of merle dogs (m/M) and two copies of this SINE insertion in "double merle" dogs (M/M) (Clark et al. 2006). In addition, Clark et al. identified "cryptic" merle dogs with a significantly shorter SINE insertion that did not display any of the classic merle phenotype (Clark et al. 2006).

PMEL plays a key role in the development of the melanosome pigment organelle within pigment-producing melanocytes in multiple species (reviewed in Theos et al. 2005). *PMEL* creates a fibrillar matrix upon which eumelanin pigment can be deposited and polymerize forming striated sheets. As the eumelanin builds, the melanosome matures into the darkly pigmented, ellipsoidal structure characteristic of the mature melanosome. No diseases have been directly associated with mutations of *PMEL* in humans or in species other than dogs, but it is thought that some forms of albinism may be linked to *PMEL* deficiency (Kerje et al. 2004). However, the wide range of ophthalmologic and auditory

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abnormalities observed in dogs with one or two copies of the *PMEL* mutation is reminiscent of features seen in human Waardenburg syndrome (reviewed in Pingault et al. 2010).

In recent years, we and other groups have correlated various lengths of the *PMEL* SINE insertion mutation with variations in the expression of the merle phenotype and established methods for routine genetic testing of merle allele sizes (Ballif et al. 2018; Murphy et al. 2018; Langevin et al. 2018; Pelles et al. 2019; reviewed in Varga et al. 2020). These studies have demonstrated that, in general, the size of the SINE insertion correlates well with predictable phenotypic expression. Based on an analysis of *PMEL* alleles in merle Australian shepherds and related breeds, we previously identified a spectrum of merle phenotypes that can be separated into at least four major phenotype clusters based on *PMEL* allele sizes that we termed "cryptic" (M200—246 bp), "atypical" (M247—264 bp), "classic" (M265—269 bp), and "harlequin" (M270—280 bp). In general, a single copy merle variant in the cryptic size range will result in a non-merle phenotype, atypical merle variants most commonly result in a "dilute" phenotype, classic merle variants produce traditional merle phenotypes, and harlequin merle variants can result in a range of phenotypes from a patchwork of multiple shades of the same or different colors with or without white to subtle almost non-merle appearances. Furthermore, we and others have also identified numerous cases of mosaicism in dogs of various breeds including cases of gonadal mosaicism (Ballif et al. 2018; Murphy et al. 2018; Langevin et al. 2018; Pelles et al. 2019; reviewed in Varga et al. 2020). In addition, several cases of merle insertion expansion and contraction have also been observed (Ballif et al. 2018; Murphy et al. 2018; Langevin et al. 2018; Pelles et al. 2019; reviewed in Varga et al. 2020). Thus, with all of this in mind, routine genetic testing can now be used to facilitate appropriate breeding strategies to avoid producing puppies with health risks associated with "double merle" dogs while maintaining desirable merle coat patterns.

The dachshund is one of many breeds that displays the merle phenotype which, in dachshunds, is commonly referred to as "dapple". In this study, we analyzed the *PMEL* gene in 161 dachshunds and correlated the allele sizes of merle SINE insertions with the phenotype of the coat in 140 dogs. Our results demonstrate that, in general, the allele sizes correlate well with the major phenotypic clusters previously identified in Australian shepherds and other breeds with only slight variation at the transition zones between clusters. However, this variation in the size of the SINE insertions observed in some phenotype clusters suggests that the genotype–phenotype clusters may vary slightly between breeds, perhaps due to their specific genetic backgrounds, and that certain size ranges may be more prevalent in some breeds due to selective breeding strategies. In addition, specific examples within our cohort of dachshunds illustrate some important points related to cryptic, hidden, and mosaic merle alleles that should be considered by all breeders of merle/dapple dogs to avoid producing puppies with serious health conditions.

Methods

Sample acquisition and DNA extraction

Samples from 161 dachshunds were collected as part of a larger study of the impact of canine *PMEL* genotype on coat color/pattern phenotype in various breeds. Of the 161 dogs analyzed in this study, 80 samples came from a large, six-generation pedigree. The remaining 81 samples were obtained through recruitment from breeders and owners with dogs displaying specific phenotypes or from otherwise-discarded DNA samples after clinical testing at Paw Print Genetics. Therefore, this cohort is not a representative cross section of the entire dachshund breed and does include many related dogs. Breeders and owners self-identified their dogs as dachshunds. Samples were obtained primarily from buccal swabs and whole blood. DNA was extracted using routine, standard methods as previously described (Shaffer et al. 2015, 2016, 2017).

PMEL SINE insertion size analysis

PCR amplification of the region flanking the SINE insertion site in *PMEL* was performed with two different primer sets to verify and confirm the results using an Applied Biosystems SeqStudio Genetic Analyzer. SINE insertion size analysis and mosaicism estimations for each sample were performed using in-house genotyping software that was developed and validated as previously described (Ballif et al. 2018).

Results

Genotype–phenotype correlation in dachshunds with a single SINE insertion

Of the 161 dogs tested, 102 were found to have a single copy (m/M) of the SINE insertion; 14 had two copies of the SINE insertion (M/M); 18 had two copies of the SINE insertion plus a normal non-merle (wild type, m) allele (m/M/M); 6 had three copies of the SINE insertion and no non-merle allele (M/M/M); and 21 were non-merle (m/m). We identified a total of 184 SINE insertions in 140 dachshunds (Fig. 1). Some dogs had one or two SINE insertions, while others were mosaic for more than one merle allele.

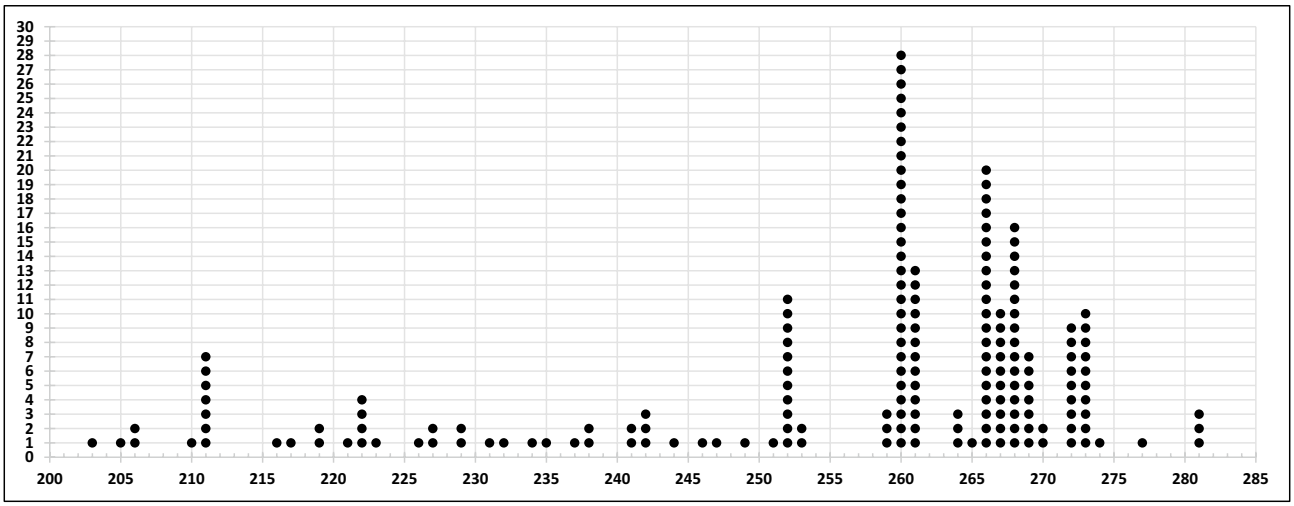

Fig. 1 *PMEL* insertion sizes ($N=184$) identified in 140 dachshunds. Each black dot indicates the size of the SINE insertion(s) present in each dog analyzed. Because some dogs had more than a single SINE insertion, the total number of SINE insertions ($N=184$) is larger than the number of dogs identified with at least one merle insertion ($N=140$)

The SINE insertions ranged from 203 to 281 base pairs (bp). To evaluate the impact of SINE insertion size on the dachshund merle/dapple phenotype, we performed a genotype–phenotype correlation with the 102 dachshunds that had a single merle allele (m/M).

In general, genotype–phenotype correlation identified the same four broad phenotypic clusters that we had previously identified in Australian shepherds (Ballif et al. 2018) and designated as cryptic, atypical, classic, and harlequin merle (Fig. 2). As with Australian shepherds and other breeds, the smaller "cryptic" merle alleles tend to have no impact on the coat color or pattern. Because no dogs with single merle alleles in the range of 243–248 bp were identified in this cohort, we could not further refine a specific size

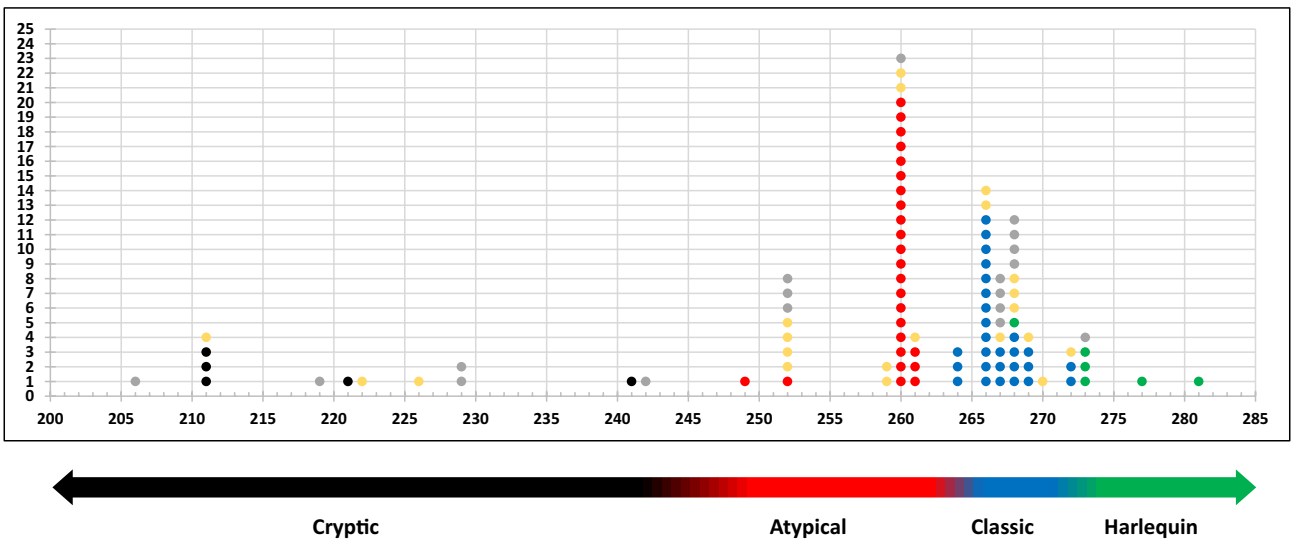

Fig. 2 Genotype–phenotype comparison of 102 dachshunds with a single copy of the SINE insertion. The dot plot shows the location of each dog's SINE insertion and their corresponding coat color group. Black dots indicate dogs with no merle/dapple phenotype, red dots indicate dogs with atypical merle/dapple coat colors such as "faux dilute", blue dots indicate dogs with classic merle/dapple coats, green dots indicate dogs with "faux harlequin" merle/dapple coats, and gray dots indicate dogs for which phenotypic information was not available. Yellow dots indicate "hidden" merle/dapple where the coat color of the dog was a light or reddish shade making it difficult or impossible to detect the merle/dapple pattern. SINE insertion size ranges of the four major phenotypic groups are identified along the heat map at the bottom which shows the transition zones between clusters as a blended color, indicating the potential for either phenotype in the transition zones (see Table 1)

at which the transition from "cryptic" to "atypical" occurs. Dachshunds with merle variants in the "atypical" size range tend to have dilute coat colors similar to what has been identified in Australian shepherds and other breeds. These "atypical" dilute dachshunds are often referred to as "faux dilute dapple" to distinguish them from coat color dilution associated with mutation of *MLPH* (D Locus, Drögemüller et al. 2007). The transition from the "atypical" to "classic" merle phenotype in these dachshunds can be narrowly defined to insertions between 262 and 263 bp. Interestingly, three dachshunds with classic merle appearance had insertions of 264 bp, which, based on our previous studies in Australian shepherds, would have placed these dogs in the "atypical" cluster. The transition from the "classic" to "harlequin" phenotype is not clear in our cohort, in that one dog with a patchwork/harlequin coat was identified to have an insertion of 268 bp which overlaps with the "classic" merle cluster (classic ranges from 264–269 bp in dachshunds). These "harlequin" dachshunds are often referred to as "faux harlequin dapple" to distinguish them from the true harlequin pattern found in great danes (Clark et al. 2011). Several other "harlequin" dogs had insertions of 273 bp or larger. These data suggest that the boundaries between phenotypic groups are not discrete, but rather transition zones with some breed-specific variation. Table 1 shows a summary of the approximate size ranges and general phenotypes for the four major groups identified in this study.

Genotype–phenotype correlation in dachshunds with two SINE insertions

We identified 14 dachshunds with two SINE insertions (M/M). Table 2 lists the sizes of the SINE insertions identified in these dogs and their phenotypes. These data indicate that the larger insertion is usually an indicator of the overall phenotype. Interestingly, we identified one dog with two insertions in the classic range (M268/M268) that looked classic merle with no obvious double merle phenotype, with the exception of a split white face. No hearing or visual impairments were observed by the owner in this dog. All ten puppies sired by this dog have had classic merle/dapple presentations, consistent with this dog being homozygous for the M268 allele (Fig. 3).

Genotype–phenotype correlation in dachshunds with mosaicism

We identified 24 dachshunds that were mosaic with more than two *PMEL* alleles. Table 2 lists the *PMEL* alleles identified in these dogs as well as the sizes of the SINE insertions identified and their phenotypes. Eighteen of these dogs were identified as having one non-merle allele (m) and two M alleles characterized by two different-sized SINE insertions. Six other dogs had three different M alleles and no non-merle allele indicating that they were mosaic for different M insertion alleles. Once again, the largest insertion variant tended to be most predictive of the overall phenotype of the dog. However, the percentage of cells impacted by each variant can also contribute to the phenotype.

Identification of gonadal mosaicism and inheritance of unstable alleles leading to mosaicism

Figure 4 shows an example of gonadal mosaicism identified in a female from a large six-generation dachshund pedigree containing more than 80 dogs. This female was genotyped as mosaic for three different-sized SINE insertions (mos M261[49]/M266[37]/M211[14]) corresponding to three different phenotype clusters (cryptic, atypical, and classic). Bracketed numbers are an estimate of relative percentage of mosaicism for each SINE insertion (Ballif et al. 2018). Figure 4a illustrates how this single mosaic female parent

Table 1 SINE insertion size ranges and phenotypic summaries for the dachshund compared to Australian shepherds and related breeds

SINE insertion size (bp)	Merle phenotype group	Phenotype summary
No insertion	Non-merle/dapple	No merle/dapple, wild type
200–246	Cryptic	Most cases are non-merle/dapple but in rare cases may have very small merle/dapple patch(es) or subtle color anomalies
247–**263**	Atypical	Most cases show a significant shift from normal coat color, often giving a diluted color, show reddish undertones, or have an otherwise atypical merle/dapple appearance. Often referred to as "faux dilute dapple" in dachshunds
264–269	Classic	Most cases show classic merle/dapple with a significant amount of merle/dapple color and patterning, although some cases may only show a minimal amount of merle/dapple
> 270	Harlequin	Most cases display patches of multiple shades of the same or different colors without white (tweed) or with white (harlequin) and is often referred to as patchwork or "faux harlequin dapple" depending on the breed. Some dogs may even be non-merle/dapple or may have very small dapple patch(es) or subtle color anomalies

Numbers highlighted in bold indicate differences from the sizes and groups published in Ballif et al. (2018) for Australian shepherds and related breeds. The size range for the group was not modified if there were no merle alleles identified near the boundaries in the dachshunds tested

Table 2 Genotype–phenotype comparison of dogs with two SINE insertion variants (M/M) and dogs with mosaic SINE insertion variants (mos m/M/M and mos M/M/M)

M locus genotype	M locus phenotype
Non-mosaic with two SINE insertions	
M211/M260	Atypical
M211/M261	Atypical
M242/M242	Atypical[a]
M244/M251	Atypical
M235/M253	Hidden dapple
M222/M268	Classic
M259/M273	Classic
M261/M266	Classic
M252/M272	Harlequin
M252/M272	Harlequin
M253/M268	Harlequin
M267/M267	Double dapple
M268/M268	Classic[b]
M269/M281	Double dapple
Mosaic with one normal (non-dapple) allele	
mos m/M260[85]/M247[15]	Atypical
mos m/M260[93]/M237[7]	Atypical
mos m/M261[63]/M216[37]	Atypical
mos m/M261[89]/M238[11]	Atypical
mos m/M261[95]/M246[5]	Atypical
mos m/M210[84]/M266[16]	Classic
mos m/M272[95]/M203[5]	Classic
mos m/M273[82]/M206[18]	Classic
mos m/M273[90]/M217[10]	Classic
mos m/M273[94]/M252[6]	Classic
mos m/M273[93]/M238[7]	Harlequin
mos m/M260[54]/M272[46]	Hidden dapple
mos m/M260[55]/M272[45]	Hidden dapple
mos m/M266[90]/M234[10]	Hidden dapple
mos m/M274[87]/M231[13]	Hidden dapple
mos m/M281[83]/M227[17]	Cryptic[c]
mos m/M273[93]/M222[7]	Not available
mos m/M272[89]/M219[11]	Not available
Mosaic with no normal (non-dapple) alleles	
mos M261[49]/M266[37]/M211[14]	Atypical[d]
mos M261[55]/M265[43]/M227[2]	Atypical
mos M261[56]/M266[37]/M223[6]	Atypical
mos M269[44]/M269[44]/M232[12]	Classic
mos M261[41]/M222[31]/M266[28]	Harlequin
mos M241[56]/M270[37]/M205[7]	Not available

Bracketed numbers are an estimate of relative percentage of mosaicism for each SINE insertion (Ballif et al. 2018)

[a]This dog showed a dilute "atypical" phenotype in spite of carrying two copies of the M242 allele which is in the "cryptic" size range

[b]Although this dog showed a "classic" merle phenotype (with the exception of a split white face, see Fig. 3), no non-merle allele was detected. Furthermore, all ten puppies sired by this dog have had "classic" merle phenotypes

[c]Cryptic "harlequin" (see Fig. 6)

[d]Gonadal mosaic (see Fig. 4)

Fig. 3 Photograph of male dachshund genotyped as an M268/M268 "double merle/dapple" with none of the classic double merle features. The split white face appears to be the extent of the double merle phenotype, although split face can occur on other coat color backgrounds and might not be related to a double dose of M268. This dog has sired ten puppies, all of which have been classic merle/dapple

contributed each of her three M alleles to individual offspring across three different pairings with non-merle sires. Figure 4b shows examples of how the pups that inherited each of the different alleles display the expected phenotype. Thus, the presence of mosaicism in a dog should be an important consideration when determining breeding practices as gonadal mosaicism may result in a dog being able to pass on more than two different alleles to its offspring.

Examples of hidden and cryptic merle in dachshunds

Dachshunds, like many breeds, carry variants at the E, K, and A coat color loci that can result in coats without eumelanin such as cream, red, or sable. In dachshunds, a pure red or cream phenotype can be a result of an "e/e" genotype at the E locus, whereas some "red" and "sable" colors are a result of agouti gene expression (ky/ky at the K locus and Ay/* at the A locus, where *denotes any A locus allele). Because these dogs do not produce eumelanin, or only minimal amounts, in their coat, the impact of merle is "hidden" in these dogs. This is an important consideration in dogs with a light pheomelanin coat as breeding a "hidden" merle carrier to a known merle dog could result in a double merle/dapple offspring with serious health deficits. We found many examples of "hidden" merle alleles of a variety of sizes in our dachshund cohort (Fig. 2). Figure 5 shows two examples of dogs with light-colored coats and "hidden" atypical and classic merle variants.

Although "cryptic" merle is most commonly associated with much shorter SINE insertions, some dogs with SINE insertions in the harlequin size range have been reported to have minimal visible merle (Langevin et al. 2018). Figure 6 demonstrates an example of "cryptic" merle male dachshund with a *PMEL* insertion in the harlequin size range. Because this dog appeared to be a solid black and tan dachshund with

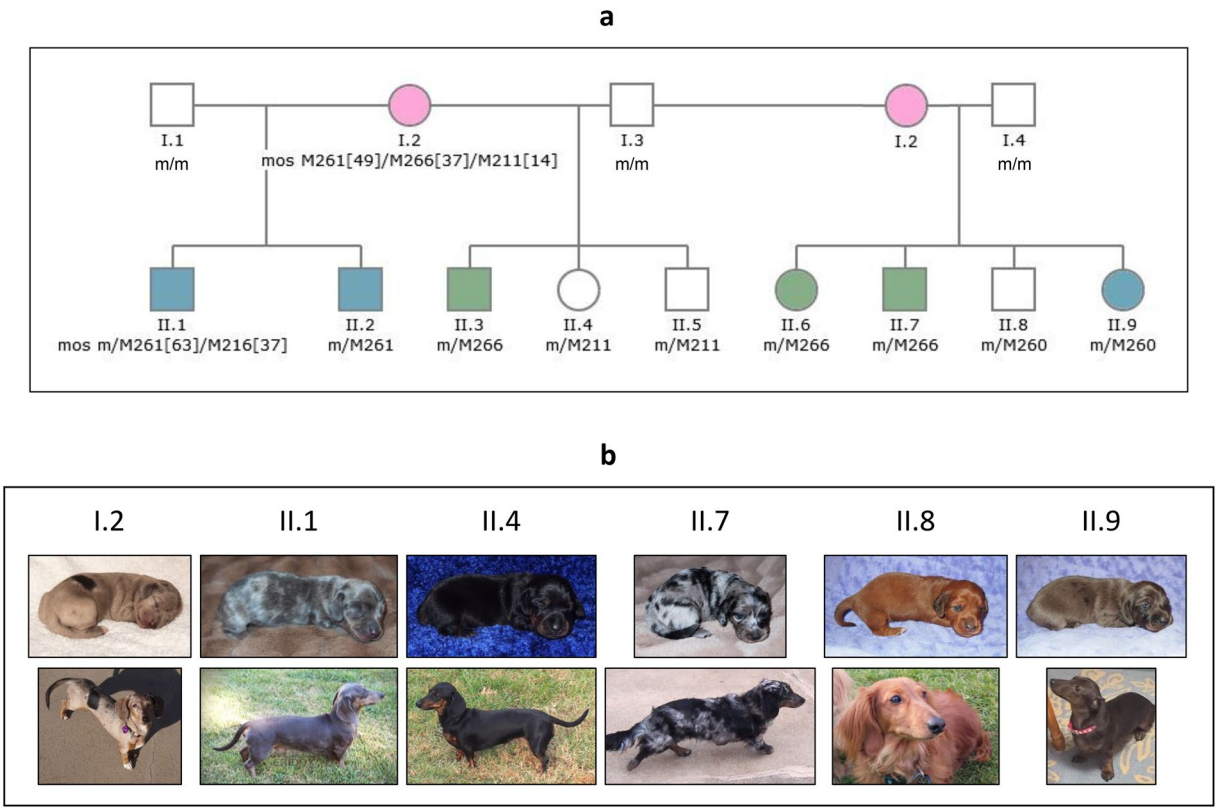

Fig. 4 Example of gonadal mosaicism in a female dachshund who transmitted three unique SINE insertions to her offspring. **a** Dam (I.2) was bred to three different, non-merle/non-dapple males (I.1, I.3, and I.4) that were tested as m/m. The genotypes of the dam, sires, and offspring are shown. The dam tested as mosaic for three different alleles of *PMEL* (M261, M266, and M211). The genotypes detected in the offspring suggest that the dam passed the M261 allele to puppies II.1, II.2, II.8, and II.9; the M266 allele to puppies II.3, II.6, and II.7; and the M211 allele to puppies II.4 and II.5. The single base pair difference in a few puppies is within the range of the assay resolution (± 1 bp) as previously reported (Ballif et al. 2018). **b** Representative images (puppy images on top and corresponding adult images on bottom for each dog) of the phenotypes for puppies produced by I.2 with each of three different alleles being represented. The dam (I.2) is mostly atypical with a large black spot on her back. II.1 has an atypical "faux dilute" phenotype. II.4 has a cryptic merle phenotype. II.7 has a classic merle/dapple phenotype. II.8 has an atypical merle mutation size, but it is difficult to see any dilution on the red pheomelanin base coat. II.9 has an atypical "faux dilute" phenotype

no visible phenotypic expression of merle, he was bred to a classic merle female. The subsequent litter resulted in four pups with non-merle (m/m), merle (m/M269), harlequin merle (m/M281), and double merle (M269/M281) phenotypes. Unfortunately, the double merle pup was diagnosed with bilateral anophthalmia, but no known hearing deficits (although extensive hearing testing was not performed). Unlike cryptic merle alleles with relatively small SINE insertions, this "cryptic" harlequin allele was phenotypically undetected in the parent, but was in the size range to produce a double dapple phenotype in the pup.

Transmission of merle alleles in multi-generation pedigrees

To evaluate the stability of merle allele transmission from one generation to the next, we evaluated the merle genotypes present in a six-generation dachshund pedigree with more than 80 dogs. Within this pedigree, there were 24 pairings involving parents with merle alleles of various sizes. In those 24 pairings, a merle allele was passed from parent to offspring 53 times. Of those 53 transmission events, the merle allele was stably transmitted from parent to offspring 100% of the time with no significant variation in allele size (data not shown). Alleles from

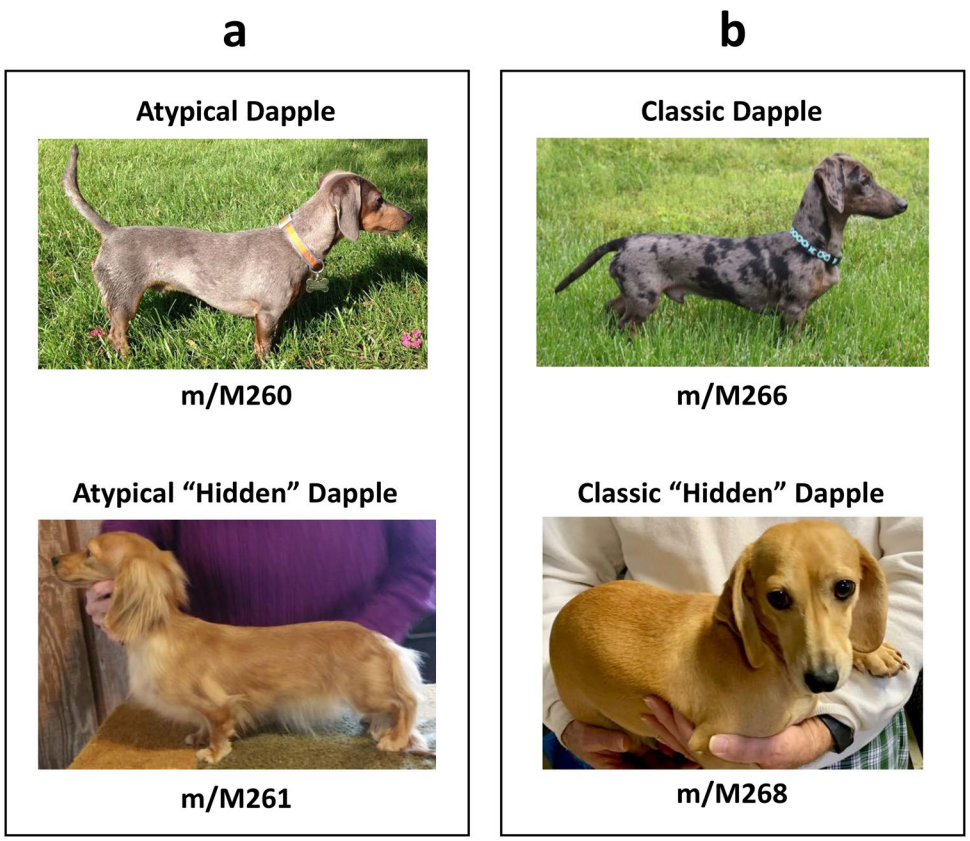

Fig. 5 Comparison of dogs with similar SINE insertion sizes on dark versus light coat colors demonstrates challenges associated with visually identifying "hidden" merle/dapple in light coated dogs. **a** Comparison of dogs with dark (top) and light (bottom) coat colors and SINE insertions in the "atypical" size range. SINE insertions in the "atypical" size range can have a dilution effect on the coat color as demonstrated in the dog with the dark coat. It is difficult to determine the impact of an insertion in the "atypical" size range for dogs with a light coat, thus making it a "hidden" merle/dapple. **b** Comparison of dogs with dark (top) and light (bottom) coat colors and SINE insertions in the "classic" size range. Insertions in the "classic" size range in a dog with a light-colored coat do not show a phenotypic impact making them true "hidden" merle/dapple

offspring that were ± 1 bp of the parental allele were considered to be stably transmitted within the resolution of the assay. Although no germline expansions or contractions of the parental alleles were observed in this pedigree, 8 of the 53 dogs evaluated (~ 15%) were found to be mosaic from buccal swabs or blood samples for another, smaller merle insertion suggesting that somatic contraction had occurred at some point during development (data not shown).

A separate, multi-generation pedigree involving 14 dogs is illustrated in Fig. 7. This pedigree also demonstrated stable meiotic transmission of an M273 allele across four generations with some occurrences of somatic mosaicism. However, photographs of dogs from this pedigree carrying the same harlequin allele inherited across three generations illustrates the variability in the phenotypic presentation of the same merle insertion (Fig. 7b and c). This is likely due to some differences in genetic background even between related dogs such as base coat color genes or modifying variants such as the S locus white spotting allele (Karlsson et al. 2007) as well as the randomness of the merle presentation due to somatic mosaicism during development (reviewed in Varga et al. 2020).

Discussion

The identification of a spectrum of merle coat color phenotypes in the Australian shepherd (Ballif et al. 2018) led us to ask if the same-sized merle alleles would produce similar phenotypes in other breeds. We therefore examined the merle alleles of 161 dachshunds and correlated the genotypes with the phenotypes in this breed. As part of this study, we evaluated the merle insertions and their subsequent inheritance in a large, six-generation pedigree containing more than 80 dogs and a smaller, four-generation pedigree containing 14 dogs.

The results of this study suggest that, in general, the previously identified merle allele clusters for Australian shepherds hold across the genotype–phenotype spectrum for dachshunds (Fig. 2 and Table 1). As we were not able to identify dachshunds with every possible allele size, precise boundaries could not be identified for all phenotypic groups. However, some variation was observed in that classic merle dachshunds frequently had mutations in the 264 bp range. which, based on our previous studies of Australian shepherds, would have placed dogs with this size allele in the "atypical" category. Although one

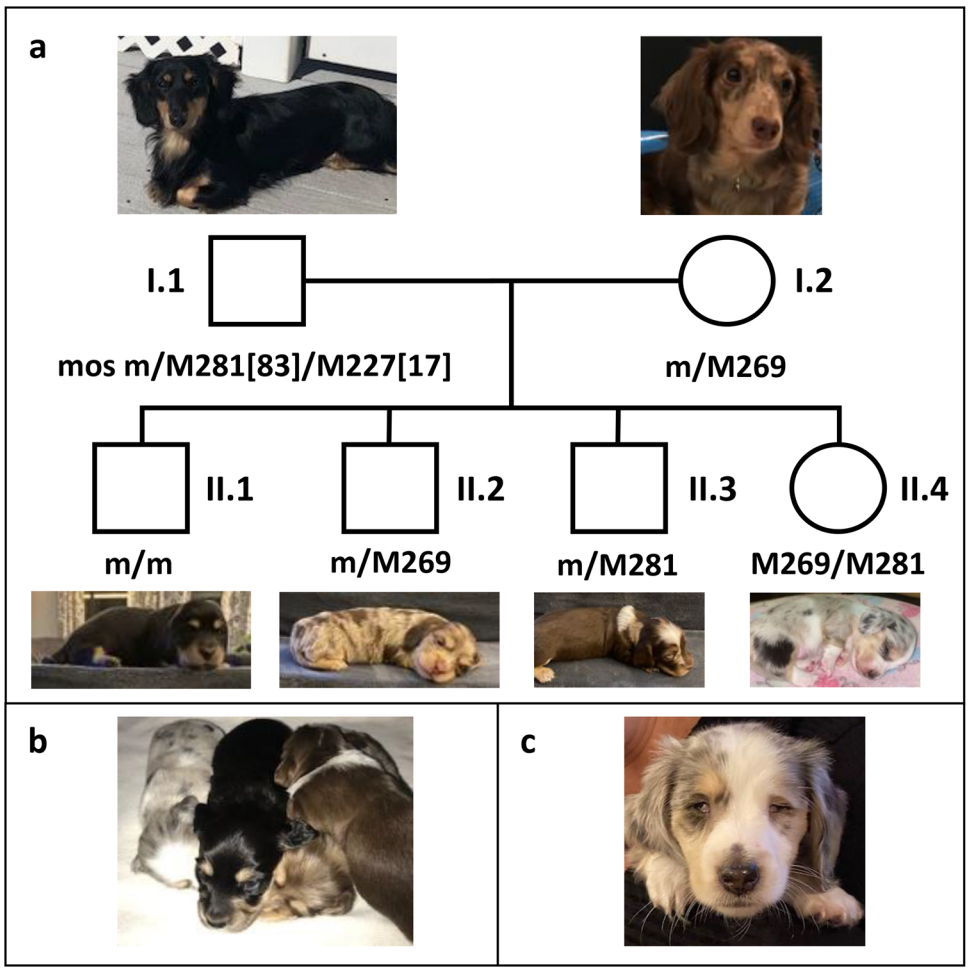

Fig. 6 "Cryptic" harlequin allele resulting in double merle/dapple pup. **a** Sire (I.1) showing typical black and tan phenotype was bred to a classic merle female (I.2) without genetic testing being performed on the sire. The subsequent litter produced by this pairing consisted of four pups including a double merle/dapple pup with bilateral anophthalmia (II.4). Genotyping identified the sire to be a carrier of an allele in the harlequin size range, that had a cryptic phenotype (mos m/M281[83]/M227[17]). Photographs of each dog indicate that the genotype correlates well with the observed phenotype for each pup and the dam. **b** Siblings shown side by side demonstrate the distinct color differences associated with each pup's genotype. **c** Double merle/dapple pup with bilateral anophthalmia

base pair may not be statistically significant in such a small collection of dogs that are mostly related to each other, it does further support that there are transition zones between phenotypic groups rather than discrete boundaries. This may also indicate that there are breed-specific genetic factors contributing to merle phenotypic expression that only become apparent in the transition zones and that studying this phenomenon by breed has merit. As merle allele data becomes more readily available for a variety of breeds, we may discover that there are other, subtle differences between breeds. Interestingly, our data demonstrate that dogs inheriting the same allele passed down through three generations do not always display the same phenotypic pattern as the parent, even though the merle insertion is of the same size. This further illustrates the variability in the expression of the merle allele which can be impacted by other factors such as genotypes at other coat color genes and somatic mosaicism. Although hidden and cryptic insertions as well as mosaicism have been previously described by us and others, these types of variants were all identified in this particular cohort of dachshunds and illustrate several important points that are worth emphasizing again.

First, a "hidden" merle refers to a dog that carries a merle allele that, in most situations, would be phenotypically visible, but has been hidden because the dog has a primarily pheomelanin-based, light-colored coat. Primarily, light-colored coats expressing little to no eumelanin effectively hide the presence of a merle allele (Fig. 5). Thus, visual inspection of the dog alone may not provide any indication that the dog is a carrier of a merle allele. This phenomenon has also been observed by us in other breeds, such as fawn-colored French bulldogs, Chihuahuas, and Boston terriers (data not shown). Breeding a "hidden" merle dog to a classic merle dog could result in a double merle pup with serious health issues. Thus, it is recommended that light-colored dogs be tested for merle before breeding to a classic merle dog.

Second, "cryptic" merle dogs typically refer to dogs that carry a merle allele that is too small to produce any phenotypic effect. Interestingly, one dog in this cohort of dachshunds did not have any visible merle or harlequin merle

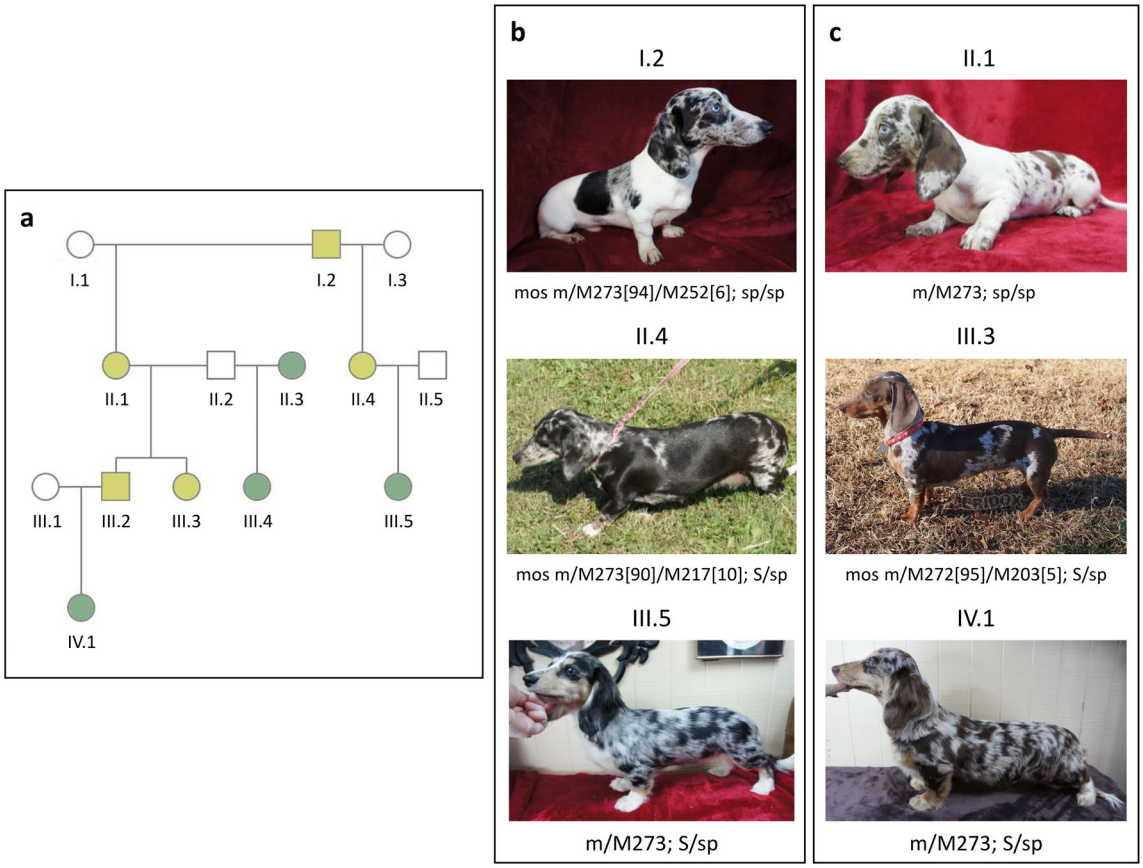

Fig. 7 Four-generation dachshund pedigree illustrating stable transmission of merle alleles but variability in phenotypic expression. **a** Four-generation pedigree with 14 dogs. No fill indicates a non-merle/non-dapple dog; light green indicates the dog has a harlequin merle/dapple phenotype; and dark green indicates dogs that appear to have more of a classic merle/dapple phenotype. **b, c** These panels illustrate a three-generation pedigree and the genotypes and phenotypes of dogs with the same M273 allele as it is passed from one generation to the next. The photographs on the top of the panel represent the first generation, the middle set of photographs show the second generation, and the photographs on the bottom represent the third generation. S locus gentoypes are also shown

phenotype, but was found to carry an insertion in the harlequin size range (Fig. 6). Although dogs with harlequin merle allele sizes have been documented previously to show little to no phenotypic change (Langevin et al. 2018), it is worth highlighting here because this dog was assumed to be non-merle or perhaps carrying only a cryptic merle allele based on the phenotype and was bred to a classic merle dog. Unfortunately, this pairing led to a double merle pup with serious health issues. Therefore, one cannot assume that because a dog has a dark base coat and a non-merle phenotype that the dog is either a non-merle or has a cryptic merle variant.

Although expansion and contraction of merle alleles have been documented (Ballif et al. 2018; Murphy et al. 2018; Langevin et al. 2018; Pelles et al. 2019), with contractions being much more frequently observed than expansions, there is still very little data to provide any good estimates of the frequency with which these events might occur in the germline. The most controversial point in this is: is it safe to breed a cryptic merle dog to a classic merle dog? Or, in other words, how often do expansions of small alleles in the cryptic range occur that could lead to unintentionally producing a double merle pup when breeding a cryptic merle dog to a classic merle dog. Although expansions and contractions are clearly rare, there is very limited data available in the scientific literature to allow for an accurate estimation of the frequency or relative risk. Protecting animal health is paramount, and if there is a chance of producing an affected double merle pup, is it worth the risk, even if the risk is extremely low? In contrast, with the prevalence of alleles of all sizes across the cryptic range and the relative risk assumed to be extremely low, would avoiding breeding cryptic merle dogs to classic merle dogs unnecessarily restrict the gene pool? This is likely not a concern for breeds such as Australian shepherds and dachshunds, but it could be a concern in other breeds.

In addition, there is a stigma associated with producing double merle pups, so reporting and documenting these types of already extremely rare events remains challenging.

In this study, we evaluated a large pedigree of more than 80 dogs across six generations in an attempt to document the transmission of merle alleles and see if we could identify and/or quantify the frequency of any significant contractions or expansions. Interestingly, of the 24 pairings involving parents with merle alleles of various sizes, we were able to document stable transmission of the merle allele from parent to offspring in 53/53 (100%) of the parent to child transmission events. Given that all merle alleles were transmitted faithfully from one generation to the next with no expansions or contractions detected in the main parent allele, we can conclude that these types of events may be quite rare. However, the 53 events evaluated here is still a relatively small number, and much larger studies will need to be performed to truly determine the frequency with which expansions and contractions actually occur. It is important to note that even though germline mosaicism was not detected in those 53 events, mosaic cell populations were found in about 15% of cases suggesting that at some point a somatic change occurred (primarily contractions of the parent allele), resulting in mosaic cell lines with much smaller allele sizes compared to the parent allele. These data seem to suggest, once again, that contractions are much more common than expansions. Combining these results with those previously documented for expansions and contraction (Ballif et al. 2018; Murphy et al. 2018; Langevin et al. 2018; Pelles et al. 2019; reviewed in Varga et al. (2020), the available data suggest that the risk of producing a double merle dog due to a cryptic merle allele expansion is extremely low but not zero.

Finally, the generation of mosaic cell lines in some dogs early in development can lead to gonadal mosaicism which has important implications for breeding. Determining which dogs with detectable somatic mosaicism through genotyping that are actually gonadal mosaic can only practically be determined by testing offspring (Ballif et al. 2018; reviewed in Varga et al. 2020). Although gonadal mosaicism has also been documented previously (Ballif et al. 2018; Murphy et al. 2018; Langevin et al. 2018) and is presumed to be rare, it is not clear how common this type of an event might be. In our cohort of dachshunds, we identified only one mosaic female that was passing three different merle alleles to her offspring, each with different phenotypic expression (Fig. 4). Although we identified numerous other dogs with mosaicism, it is unclear if these dogs are also gonadal mosaic. We were only able to determine that gonadal mosaicism was present in the one female dog by genotyping all of her offspring across three different litters. Breeders should keep in mind the possibility of gonadal mosaicism when considering a breeding with a dog that has been genotyped as mosaic.

One other dog with an unexpected and potentially gonadal mosaic genotype is worth mentioning here in that he appeared phenotypically classic merle aside from a large white patch on his face (Fig. 3). However, genotyping showed the dog to be a double merle with two merle alleles in the classic range, and all ten of this dog's offspring have been classic merle. Although this dog could have a different genotype in its gonads compared to other parts of its body, this would need to be confirmed by additional testing.

The data presented in this study of merle (dapple) in the dachshund illustrates a wide range of complexities associated with merle variants that need to be considered by breeders of dogs that may have merle in their lines. The potential for hidden, cryptic, and mosaic (germline and somatic) merle alleles demonstrate the critical need for breeders to consider genetic testing prior to breeding, although testing of somatic tissues may not always reveal the possibility of gonadal mosaicism. Now that high-resolution genetic testing for merle allele size has become widely available at relatively low cost, testing breeding dogs for the presence of merle variants can provide valuable information needed to make the most informed breeding decisions and avoid producing puppies with serious health issues due to this mutation.

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Authors' contributions LGS, BCB, and LJE contributed to the study conceptualization and design. Sample recruitment, photo collection, and pedigree chart construction were performed by LJE for the large six-generation pedigree. *PMEL* testing, laboratory supervision, and results analysis was performed by HFS, CJR, CRC, KS, and BCB. BCB performed the final analysis of the combined dataset and wrote the first draft of the manuscript. All authors commented on and approved the final manuscript.

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Availability of data and material All relevant data generated in this study are included in this published article.

Code availability Not applicable.

Declarations

Conflict of interest LGS is the owner of Genetic Veterinary Sciences, Inc., DBA Paw Print Genetics which provides genetic testing on a fee-for-service basis. The remaining authors have no conflicts of interest to declare.

Ethics approval Informed consent was obtained from the owners of dogs specifically recruited for this study. The remaining samples were anonymized from samples that would have been otherwise discarded following routine diagnostic testing.

Consent to participate All canine samples included in this study were obtained through consent of the individual owners or were obtained from otherwise-discarded DNA samples after clinical testing at Paw Print Genetics.

Consent for publication All photographs included in this study were used with permission from the dog owners.

References

Ballif BC, Ramirez CJ, Carl CR, Sundin K, Krug M, Zahand A, Shaffer LG, Flores-Smith H (2018) The PMEL gene and merle in the domestic dog: a continuum of insertion lengths leads to a spectrum of coat color variations in Australian shepherds and related breeds. Cytogenet Genome Res 156:22–34. https://doi.org/10.1159/000491408

Clark LA, Wahl JM, Rees CA, Murphy KE (2006) Retrotransposon insertion in SILV is responsible for merle patterning of the domestic dog. Proc Natl Acad Sci USA 103:1376–1381. https://doi.org/10.1073/pnas.0506940103

Clark LA, Tsai KL, Starr AN, Nowend KL, Murphy KE (2011) A missense mutation in the 20S proteasome β2 subunit of Great Danes having harlequin coat patterning. Genomics 97:2440248. https://doi.org/10.1016/j.ygeno.2011.01.003

Drögemüller C, Philipp U, Haase B, Günzel-Apel AR, Leeb T (2007) A noncoding melanophilin gene (MLPH) SNP at the splice donor of exon 1 represents a candidate causal mutation for coat color dilution in dogs. J Hered 98:468–473. https://doi.org/10.1093/jhered/esm021

Gelatt KN, Powell NG, Huston K (1981) Inheritance of microphthalmia with coloboma in the Australian shepherd dog. Am J Vet Res 42:1686–1690

Karlsson EK, Baranowska I, Wade CM, Salmon Hillbertz NH, Zody MC, Anderson N, Biagi TM, Patterson N, Pielberg GR, Kulbokas EJ 3rd, Comstock KE, Keller ET, Mesirov JP, von Euler H, Kampe O, Hedhammar A, Lander ES, Andersson G, Andersson L, Lindblad-Toh K (2007) Efficient mapping of mendelian traits in dogs through genome-wide association. Nat Genet 39(11):1321–1328. https://doi.org/10.1038/ng.2007.10

Kerje S, Sharma P, Gunnarsson U, Kim H, Bagchi S, Fredriksson R, Schütz K, Jensen P, von Heijne G, Okimoto R, Andersson L (2004) The Dominant white, dun and smoky color variants in chicken are associated with insertion/deletion polymorphisms in the PMEL17 gene. Genetics 168(3):1507–1518. https://doi.org/10.1534/genetics.104.027995

Langevin M, Synkova H, Jancuskova T, Pekova S (2018) Merle phenotypes in dogs—SILV SINE insertions from Mc to Mh. PLoS One 13:e0198536. https://doi.org/10.1371/journal.pone.0198536

Murphy SC, Evans JM, Tsai KL, Clark LA (2018) Length variations within the merle retrotransposon of canine PMEL: correlating genotype with phenotype. Mob DNA 9:26. https://doi.org/10.1186/s13100-018-0131-6

Pelles Z, Gaspardy A, Zoldag L, Lenart X, Ninausz N, Varga L, Zenke P (2019) Merle allele variations in the mudi dog breed and their effects on the phenotypes. Acta Vet Hung 67:259–173. https://doi.org/10.1556/004.2019.018

Pingault V, Ente D, Dastot-Le Moal F, Goossens M, Marlin S, Bondurand N (2010) Review and update of mutations causing Waardenburg syndrome. Hum Mutat 31(4):391–406. https://doi.org/10.1002/humu.21211

Platt S, Freeman J, di Stefani A, Wieczorek L, Henley W (2006) Prevalence of unilateral and bilateral deafness in border collies and association with phenotype. J Vet Intern Med 20:1355–1362. https://doi.org/10.1892/0891-6640(2006)20[1355:pouabd]2.0.co;2

Shaffer LG, Ramirez CJ, Phelps P, Aviram M, Walczak M, Bar-Gal GK, Ballif BC (2017) An international genetic survey of breed-specific diseases in working dogs from the United States, Israel, and Poland. Cytogenet Genome Res 153:198–204. https://doi.org/10.1159/000486774

Shaffer LG, Ramirez CJ, Sundin K, Connell LB, Ballif BC (2015) Genetic screening and mutation identification in a rare canine breed, the Drentsche patrijshond. Vet Rec Case Rep 3:e000185

Shaffer LG, Ramirez CJ, Sundin K, Connell LB, Ballif BC (2016) Genetic screening and mutation identification in a rare canine breed, the cesky fousek. Vet Rec Case Rep 4:e000346

Theos AC, Truschel ST, Raposo G, Marks MS (2005) The silver locus product Pmel17/gp100/Silv/ME20: controversial in name and in function. Pigment Cell Res 18(5):322–336. https://doi.org/10.1111/j.1600-0749.2005.00269.x

Varga L, Lenart X, Zenke P, Orban L, Hudak P, Ninausz N, Pelles Z, Szoke A (2020) Being merle: the molecular genetic background of the canine merle mutation. Genes 11:660. https://doi.org/10.3390/genes11060660