MERLE ALLELE VARIATIONS IN THE MUDI DOG BREED AND THEIR EFFECTS ON PHENOTYPES

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A retrotransposon insertion in the SILV gene is associated with a peculiar phenotype of dog, known as a merle. It is characterised by various areas of their coat colour becoming diluted due to a malfunction in the eumelanin-producing pigment cells. Recent studies have shown that the exact size of the short interspersed element (SINE) insertion is in correlation with specific phenotypic attributes, but was not able to absolutely confine dogs to a certain colour pattern. Our study focused on the merle variations occurring in the Mudi breed. Altogether, 123 dog samples from 11 countries were tested and genotyped. The exact length of the merle alleles were determined by automated fluorescent capillary fragment analysis. The most frequent merle genotype in this Mudi sample collection was the ‘classic’ merle (m/M: 61.8%), whereas other variants, such as atypical (m/Ma and m/Ma+: 5.7%), harlequin (m/Mh: 13.8%), double merle (M/M: 0.8%) and mosaic profiles (17.9%) were also observed. The practical significance of testing this mutation is that, phenotypically, not only merle dogs are carriers of this insertion, but also the so-called hidden merle individuals (where the merle phenotype is fully covered by the pheomelanin-dominated colouration) are potentially capable of producing unintentionally homozygous ‘double merle’ progeny with ophthalmologic, viability and auditory impairments.

Key words: Dog, Mudi, merle, coat colours, SILV allele variations

The first description of this Hungarian herding breed, designated as ‘Mudi’, is from 1936 (Fényes, 1936). According to that description, merle (in Hungarian: ‘cifra’) dogs were also taking part in the foundation of the breed from the beginning. After World War II, when breeding restarted, it was assumed that the merle variety had become extinct, as none of them were found at breeders in the substantially reduced Mudi population in Hungary. The merle colour was reintroduced into the breed in 1994 by a blue merle female from a shepherd’s stock. It is assumed by some dog breeders that the original merle variation still survived

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as shepherd dogs (Wenzel, 2000). Since then, additional merle individuals with unknown origin have been added to the open studbook, but still many dogs of the current Mudi population have inherited their merle colouration from the above-mentioned female. The blue-merle colour is described in the English version of the Mudi standard as 'black speckled, estriped, -brindle or -spotted on lighter or darker bluish-grey primary colour' regarding the coat, and states 'only in blue-merle dogs, wall (white or blue) eyes are not faulty' with regard to the eyes (Fédération Cynologique Internationale, 2004; FCI-Standard No. 238). The merle pattern is observed in heterozygous individuals and can be expressed not only on black, but also on any base-colour dominated by the eumelanin pigment. Homozygous merle dogs are predominantly white, and display the characteristic pattern only on limited areas of the coat. The hypopigmentation resulting in white on the coat of double merles typically affects also the iris, resulting in blue eyes, and a high chance exists for affecting the pigment cells of the retina and inner ear as well, which may lead to ophthalmologic and auditory impairments (Gelatt et al., 1981; Strain, 1999). It is worth mentioning that the merle mutation is not uniquely responsible for blue eyes in dogs, as a recently described tandem duplication near the ALX4 gene has also been associated with blue eye colour in Siberian Huskies (Deane-Coe et al., 2018).

The genetic background behind this unique merle phenotype was discovered by Clark et al. (2006). The causative mutation is a short interspersed element (SINE) in the premelanosome protein (PMEL, formerly designated SILV – silver) gene. With this breakthrough, genetic testing of the merle mutation became a routine procedure in dog breeds carrying this mutation, as well as in Mudas in Hungary (Hédan et al., 2006; Miluchová et al., 2015; Pelles et al., 2018). However, the test, consisting of a PCR amplification and agarose gel separation, provided controversial results in some cases, as the phenotype did not correlate with the genotype, which has facilitated the development of a more precise testing procedure. Recent studies have shown that different SINE sizes are capable of generating, not only the typical merle appearance, but also the harlequin and dilute phenotypes in heterozygous configuration. In the case of cryptic merles, the short SINE form (200–246 base pairs) has no effect on the coat colour (Ballif et al., 2018; Murphy et al., 2018). It has also been found that somatic mosaicism is a common phenomenon in merle dogs, which also increases the geno- and phenotypic complexities (Langevin et al., 2018).

The aim of this study was to survey the different merle varieties in the Mudi breed and detect their effects on the phenotype, in order to assist in the preparation of well-founded breeding and mating decisions.
Materials and methods

Sample collection and classification by phenotype

This study involved a total of 123 Mudis which either showed a merle pattern or previous genetic testing verified the presence of the elongated merle allele with SINE insertion (Pelles et al., 2018). All of the participating dogs, with the exception of double merle and albino individuals, were registered by one of the main canine breeding organisations (the Fédération Cynologique Internationale, the American Kennel Club or the Canadian Kennel Club). The double merle and the albino dogs carried the overall phenotypic characteristics of the Mudi breed, and thus were identified as Mudis by the sample submitters. Either buccal swab or hair root samples were sent by their owners or breeders, or were collected by us during various cynologic events. The main sample-collection period lasted from late 2017 to early 2018. We also collected the pedigree data and photographs showing their coat colour phenotype, and asked the owners about the dogs’ eye colour. The samples were collected from a total of 11 countries (Table 1) representing seven types of the Mudi base-colours and we assigned them to six merle phenotypic categories based on the coat colour and pattern described below (see also Fig. 3, Supplementary Table 1).

Our dilute merle category had two representatives exhibiting no recognisable merle pattern and possessing similar colouration to what is called ‘ash’ in Mudis – which is a breed-specific term for blue or diluted grey. The classic merle category mainly contained dogs having the merle pattern described in the breed standard, expressed on black-, brown-, ash-, or ashbrown- (‘isabella’ or grey-brown) base colour. Unusual merles which do not fit into the other categories – such as ‘tweed’ or ‘shaded’ merles (USA4), or merles with variously contrasted patterns (Nor5) – were assigned to the classic merle category as well, as, objectively speaking, they could not have been definitively separated (Fig. 3, Supplementary Table 1). We classified those dogs to our harlequin merle category which had extended white markings, e.g. chest patch, partial or full collar, tip of the extremities, tail, snout, which are not common characteristics of the breed, and possessed minimal white merle markings on the body. Hidden merle individuals had pigmented skin and eyes but showed no merle pattern on their coat, since their white or fawn (pheomelanin-dominated yellow or recessive red) coat colour hides any kind of abnormalities in eumelanin synthesis such as the merle pattern. In the albino merle category, the dogs had non-pigmented skin and eyes. The double merle phenotype exhibited the classic merle patches on the predominantly white body.

Detection of merle alleles

Genomic DNA was isolated from buccal swabs and/or hair samples using QIAamp® DNA Mini Kit (Qiagen) following the manufacturer’s instructions. Quality and the approximate quantity of total DNA were surveyed by agarose gel.
electrophoresis using GR Green Nucleic Acid Gel Stain (Biotium). Exon 11 of the SILV gene was amplified according to the PCR method described previously (Clark et al., 2006) using 6-Fam-labelled forward primer. The PCR products were separated and analysed by capillary fragment analysis by ABI Prism 3130XL Genetic Analyzer using GeneScan™-500 LIZ™ Size Standard (ThermoFisher Scientific). GeneMapper® ID-X software version 1.4 was used for genotyping. Bin settings for the different merle allele variants were developed, following the classification recommendations by Langevin et al. (2018). The SINE insertion size was determined by subtracting the size of the wild-type allele from the size of the highest merle allele, and the outcome was rounded to the nearest integer. In the case of homozygous merles, the wild-type allele size (205 bp) was subtracted from the most intense merle allelic peak.

Table 1

The phenotypes of samples collected from different countries

| Country             | Dilute merle | Classic merle | Harlequin merle | Hidden merle | Albino merle | Double merle |
|---------------------|--------------|---------------|-----------------|--------------|--------------|--------------|
| Hungary (n = 76)    | 1            | 64            | 1               | 5            | 2            | 3            |
| USA (n = 16)        | 12           | 1             | 3               |              |              |              |
| Canada (n = 10)     | 1            | 8             | 1               |              |              |              |
| Finland (n = 7)     | 4            | 3             |                 |              |              |              |
| Norway (n = 5)      | 3            | 2             |                 |              |              |              |
| Germany (n = 3)     | 3            |               |                 |              |              |              |
| Sweden (n = 2)      |              |               |                 | 2            |              |              |
| Czech Republic (n = 1) |           |               |                 |              | 1            |              |
| Austria (n = 1)     |              |               |                 |              |              | 1            |
| The Netherlands (n = 1) |           |               |                 |              |              | 1            |
| Cyprus (n = 1)      |              |               |                 |              |              |              |
| Total               | 2            | 97            | 2               | 17           | 2            | 3            |

Results

Using high-resolution automated fragment analysis, we were also able to successfully identify the mosaic genotypes in addition to the heterozygote and homozygote double merle genotypic categories (Fig. 1). In the case of heterozygous animals, the wild-type (m) allele was the most prominent peak on the chromatogram with the expected fragment size of 205 bp. Signals of the longer merle alleles consisted of adjacent peaks with varying intensities. The most intense peak was attributed to the size of the particular allele. In certain cases the chromatogram consisted of more than two allelic variants; in these cases the group having lower peak intensities was considered to be a minor allele of a mosaic genotype.

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### Supplementary Table 1

| Code  | Base colour | Merle phenotype | Geno-type | Merle allele (SINE) size in base pair |
|-------|-------------|-----------------|-----------|----------------------------------------|
|       |             |                 |           | Mc 200–230 | Mc+ 231–246 | Ma 247–254 | Ma+ 255–264 | M 265–268 | Mh 269–280 |
| Can10 | black       | dilute          | m/Ma+     | 255         |             |             |             |             |             |
| Hun67 | black       | dilute          | m/Ma+     | 257         |             |             |             |             |             |
| Hun64 | black       | classic         | m/Ma+     | 261         |             |             |             |             |             |
| Hun71 | ash         | classic         | m/Ma+     | 262         |             |             |             |             |             |
| Hun32 | black       | classic         | m/Ma+     | 263         |             |             |             |             |             |
| Fin7  | black       | classic         | m/M       | 265         |             |             |             |             |             |
| Hun51 | black       | classic         | m/M       | 265         |             |             |             |             |             |
| Net1  | black       | classic         | m/M       | 265         |             |             |             |             |             |
| Can2  | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Can5  | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Can6  | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Fin5  | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Ger2  | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun11 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun21 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun22 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun23 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun28 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun29 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun31 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun36 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun38 | ashbrown    | classic         | m/M       | 266         |             |             |             |             |             |
| Hun40 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun41 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun45 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun52 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun53 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun54 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun63 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Hun74 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Nor2  | black       | classic         | m/M       | 266         |             |             |             |             |             |
| USA11 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| USA12 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| USA14 | black       | classic         | m/M       | 266         |             |             |             |             |             |
| Aus1  | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Can3  | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Can4  | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Can7  | ash         | classic         | m/M       | 267         |             |             |             |             |             |
| Can8  | ash         | classic         | m/M       | 267         |             |             |             |             |             |
| Fin4  | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Hun1  | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Hun19 | brown       | classic         | m/M       | 267         |             |             |             |             |             |
| Hun20 | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Hun24 | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Hun25 | brown       | classic         | m/M       | 267         |             |             |             |             |             |
| Hun35 | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Hun39 | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Hun47 | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Hun49 | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Hun56 | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Hun6  | black       | classic         | m/M       | 267         |             |             |             |             |             |
| Code   | Base colour | Merle phenotype | Geno- type | Merle allele (SINE) size in base pair |
|--------|-------------|----------------|------------|--------------------------------------|
|        |             |                |            | Mc: 200–230 | Mc+: 231–246 | Ma: 247–254 | Ma+: 255–264 | M: 265–268 | Mh: 269–280 |
| Hun61  | black       | classic        | m/M        |            |            |            |            |            |            |
| Hun68  | black       | classic        | m/M        |            |            |            |            |            |            |
| Hun7   | black       | classic        | m/M        |            |            |            |            |            |            |
| Hun8   | black       | classic        | m/M        |            |            |            |            |            |            |
| Nor4   | brown       | classic        | m/M        |            |            |            |            |            |            |
| Nor5   | brown       | classic        | m/M        |            |            |            |            |            |            |
| USA1   | black       | classic        | m/M        |            |            |            |            |            |            |
| USA3   | black       | classic        | m/M        |            |            |            |            |            |            |
| USA7   | black       | classic        | m/M        |            |            |            |            |            |            |
| USA9   | black       | classic        | m/M        |            |            |            |            |            |            |
| Ger1   | black       | classic        | m/M        |            |            |            |            |            |            |
| Hun12  | black       | classic        | m/M        |            |            |            |            |            |            |
| Hun17  | black       | classic        | m/M        |            |            |            |            |            |            |
| Hun33  | black       | classic        | m/M        |            |            |            |            |            |            |
| Hun34  | black       | classic        | m/M        |            |            |            |            |            |            |
| Hun75  | black       | classic        | m/M        |            |            |            |            |            |            |
| Hun76  | black       | classic        | m/M        |            |            |            |            |            |            |
| USA6   | black       | classic        | m/M        |            |            |            |            |            |            |
| Can1   | black       | classic        | m/Mh       |            |            |            |            |            |            |
| Cyp1   | ash         | classic        | m/Mh       |            |            |            |            |            |            |
| Hun15  | brown       | classic        | m/Mh       |            |            |            |            |            |            |
| Hun18  | black       | classic        | m/Mh       |            |            |            |            |            |            |
| Hun42  | black       | classic        | m/Mh       |            |            |            |            |            |            |
| Hun43  | black       | classic        | m/Mh       |            |            |            |            |            |            |
| Hun44  | black       | classic        | m/Mh       |            |            |            |            |            |            |
| Hun46  | black       | classic        | m/Mh       |            |            |            |            |            |            |
| Hun48  | black       | classic        | m/Mh       |            |            |            |            |            |            |
| Hun50  | black       | classic        | m/Mh       |            |            |            |            |            |            |
| Hun57  | black       | classic        | m/Mh       |            |            |            |            |            |            |
| Hun62  | black       | classic        | m/Mh       |            |            |            |            |            |            |
| Fin6   | black       | classic        | m/Mh       |            |            |            |            |            |            |
| USA8   | ash         | classic        | m/Mh       |            |            |            |            |            |            |
| Hun9   | black       | classic        | m(Mc)/M    | (217)      |            |            |            |            |            |
| Hun13  | black       | classic        | m(Mc)/M    | (220)      |            |            |            |            |            |
| Hun14  | brown       | classic        | m(Mc)/M    | (221)      |            |            |            |            |            |
| Hun26  | ash         | classic        | m(Mc)/M    | (205)      |            |            |            |            |            |
| Hun55  | black       | classic        | m(Mc)/M    | (214)      |            |            |            |            |            |
| Hun50  | black       | classic        | m(Mc)/M    | (216)      |            |            |            |            |            |
| USA4   | black       | classic        | m(Mc)/M    | 218        |            |            |            |            |            |
| USA133 | brown       | classic        | m(Mc)/M    | (229)      |            |            |            |            |            |
| Hun27  | black       | classic        | m(Mc)/(M)  | (208)      | (249)      |            |            |            |            |
| Hun73  | ash         | classic        | m(Mc)/(M)a+| (223)      |            |            |            |            |            |
| USA16  | black       | classic        | m(Mc)/(M)  | 220        |            |            |            |            |            |
| Hun59  | black       | classic        | m(Mc+)/M   | (244)      |            |            |            |            |            |
| Ger1   | black       | classic        | m(Mc+)/M   | (245)      |            |            |            |            |            |
| Hun10  | ash         | classic        | m(Mc+)/M   | (240)      |            |            |            |            |            |
| Hun37  | ash         | classic        | m(Mc+)/M   | (237)      |            |            |            |            |            |
| Hun72  | ash         | classic        | m(Mc+)/M   | (243)      |            |            |            |            |            |
| Hun5   | black       | harlequin      | m/Mh       |            |            |            |            |            |            |
| USA2   | black       | harlequin      | m/Mh       |            |            |            |            |            |            |
**Table 1 continued**

| Code  | Base colour | Merle phenotype | Genotype          | Merle allele (SINE) size in base pair |
|-------|-------------|----------------|-------------------|--------------------------------------|
| Hun70 | albino      | albino         | M/M               | 266/266                              |
| Hun69 | albino      | M/M            |                   | 267                                  |
| Hun16 | black       | double         | (Mc)/M/M          | 266/266 (224)                        |
| Hun58 | black       | double         | (Mc)/Mh/Mh/Mh     | 269/269 (228) (244)                  |
| Hun30 | brown       | double         | (Mc)/(Mc+)/Mh/Mh  | 270                                  |
| Hun4  | fawn        | hidden         | m/Ma              | 248                                  |
| Can9  | white       | hidden         | m/Ma+             | 255                                  |
| Cze1  | white       | hidden         | m/M               | 266                                  |
| Nor1  | fawn        | hidden         | m/M               | 266                                  |
| USA5  | fawn        | hidden         | m/M               | 266                                  |
| Hun65 | fawn        | hidden         | m/M               | 266                                  |
| Hun66 | fawn        | hidden         | m/M               | 266                                  |
| Fin3  | fawn        | hidden         | m/M               | 267                                  |
| Swe2  | fawn        | hidden         | m/M               | 267                                  |
| Nor3  | fawn        | hidden         | m/M               | 267                                  |
| Hun2  | fawn        | hidden         | m/M               | 268                                  |
| Fin1  | fawn        | hidden         | m/M               | 268                                  |
| Fin2  | fawn        | hidden         | m/M               | 268                                  |
| USA15 | fawn        | hidden         | m/Mh              | 269                                  |
| USA10 | fawn        | hidden         | m/(Mc)/M          | 268                                  |
| Swe1  | white       | hidden         | m/(Mc)/M          | 267                                  |
| Hun3  | fawn        | hidden         | m/(Ma)/M          | 266                                  |

**Table 2**

Six phenotypic categories with the detected numbers of different genotypes. Minor alleles are put in parentheses and a slash sign is put between alleles.

| Genotype                      | Dilute (n = 2) | Classic (n = 97) | Harlequin (n = 2) | Hidden (n = 17) | Albino (n = 2) | Double (n = 3) |
|-------------------------------|----------------|------------------|-------------------|----------------|----------------|----------------|
| m/Ma                          |                |                  |                   |                |                |                |
| m/Ma+                         | 2              | 3                | 1                 |                |                |                |
| m/M                           | 64             | 11               | 1                 |                |                |                |
| m/Mh                          | 14             | 2                | 1                 |                |                |                |
| M/M                           |                |                  |                   |                |                |                |
| m/(Mc)/(Ma)/M                 | 1              |                  |                   |                |                |                |
| m/(Mc)/Ma+                    | 1              |                  |                   |                |                |                |
| m/Mc/(M)                      | 1              |                  |                   |                |                |                |
| m/(Mc)/M                      | 7              | 2                |                   |                |                |                |
| m/Mc/(Mh)                     | 1              |                  |                   |                |                |                |
| m/(Mc+)/Ma+                   | 3              |                  |                   |                |                |                |
| m/(Mc+)/M                     | 2              |                  |                   |                |                |                |
| m/(Ma)/M                      | 1              |                  |                   |                |                |                |
| (Mc)/M/M                      | 1              |                  |                   |                |                |                |
| (Mc)/Mh/Mh                    | 1              |                  |                   |                |                |                |
| (Mc)/(Mc+)/Mh/Mh              | 1              |                  |                   |                |                |                |

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Fig. 1. A typical chromatogram for a heterozygote, a homozygote double merle and for a mosaic individual (bp – base pair; RFU – Relative Fluorescence Unit; m – wild type allele; M – merle allele; Mc+ – cryptic merle + allele).
Fig. 2. Merle fragment analysis and allele designation with bin settings for the different merle allele variants (Mc: 200–230 bp; Mc+: 231–246 bp; Ma: 247–254 bp; Ma+: 255–264 bp; M: 265–268 bp; Mh: 269–280 bp)
Fig. 3. Representative Mudis of the six phenotype groups (a–f) with the detected genotypes and with the size (bp) of Merle alleles (minor alleles are put in parentheses and a slash sign is put between alleles)
With the use of GeneMapper® ID-X software and the adequate bin settings, all detected alleles could be clearly designated by size into one of the possible allele variants (Fig. 2).

**Phenotype–genotype correlation**

To analyse the pheno- and genotype correlations, six phenotypic groups were set to classify the Mudi samples (Fig. 3).

Altogether four heterozygote, one homozygote and 11 mosaic genotypic categories were detected by the determination of the actual allele sizes of each dog (Table 2, Supplementary Table 1).

The classic merle category, to which the majority of the dogs belong, genotypically was the most diverse group as well, with three heterozygote and seven mosaic DNA-profile types. The harlequin and the dilute merle phenotypes corresponded properly to their genotypes. Mc and Mc+ alleles were found only in mosaic results. All three dogs with double merle phenotype had mosaic genotypes.

**Discussion**

**Phenotype–genotype correlation**

Before the molecular identification of the SINE insertion into the PMEL gene and elaboration of the appropriate direct genetic test, the merle genotype was assessed on the basis of the phenotype alone. Recent efforts are promoting the genetic testing of this mutation, especially in the case of hidden merle individuals. For describing the genotype, the merle locus allele nomenclature was used in this study, as introduced by Langevin et al. (2018). The allele bins from Langevin’s classification were determined by genotyping many homozygous merle individuals of different-sized merle alleles, thus the possible effect on the phenotype of each allele was evaluated in numerous combinations. As 119 of the 123 individuals examined in our study possessed a wild-type allele – the result of breeding policies used in the Mudi breed – we were not able to investigate the phenotypes caused by homozygous merles in our sample pool. The borders set between the alleles may not be as clearly defined in heterozygous forms as they appear to be when homozygous; however, they must not be entirely disregarded.

According to our findings, the classic merle phenotype is the most common in the Mudi breed; dogs exhibiting this phenotype carry the normal merle (M) allele in 77% of the cases. The fact that not all dogs from this category had a normal merle allele can be explained by the complexity of this phenotypic category (Fig. 3, bl–III). Coat length and texture can also affect the merle phenotype within the allelic range used: dogs having long curly coat with a mixture of darker and lighter hairs might be seen as ‘shaded’ merle with low contrast, but appear to be of ‘classic’ merle when shaved. Many of the merle dogs have some sorts of
‘diluted patches’, and extended diluted patches are often called ‘tweed’; however, there is no general agreement as to where the border is located between the two. The phenomenon of merle pattern darkening with the aging of the dogs also occurs in certain cases, caused by a yet unknown factor. Based on these above-mentioned considerations, the subdivision of the main categories could not be made on an objective basis. Generally, the longer merle alleles have a stronger impact on the base coat colour; however, predicting the genotype on the basis of phenotype, and vice versa, is considerably difficult as the correlation is too low between them. There were cases where minimal visible differences could be observed between the intact and diluted shades; however, these dogs were also assigned genotypically into the classic merle category. Mh and Ma+ alleles were also found in this group, Mh alleles were at the lower and Ma+ alleles at the upper border of their categories. Dogs with a mosaic genetic profile – especially where the shorter allele showed a stronger intensity on the chromatogram – often possessed the ‘shaded’ merle phenotype (see Fig. 3, bIII).

Our dilute merle category possessed two representatives and both had Ma+ alleles (with SINE sizes of 255–257 base pairs); thus, we may conclude that these slightly shortened alleles exhibit an effect not as strong as to create a merle pattern of their own. Dogs with harlequin merle colouration had longer Mh alleles (with SINE sizes of 271–273 base pairs) compared to the Mh alleles of dogs having the classic merle phenotype. While the dilute merle dogs were relatives, the harlequin merle dogs did not have common merle ancestors in their pedigrees.

The hidden merle individuals may have various merle alleles, since phenotype–genotype correlation could not be made, as these dogs cannot express merle patterns in heterozygous forms. However, the characteristic blue eye colour of the hidden merle dogs can be a phenotypic sign of the merle allele. It should be mentioned that below a certain size, the merle alleles are unable to modify the eye colour or create the characteristic coat pattern (Langevin et al., 2018). In the case of the longer merle alleles, it was a random process whether or not the original eye colour changed; however, if it did change, it followed a certain pattern (Schwab et al., 2016). Albino dogs have a non-pigmented iris, thus the eye colour may not be used to deduce the presence of the merle allele. These dogs do not exhibit the merle pattern either in homo- or in heterozygous forms. All dogs involved in our research with the double merle phenotype had mosaic genotypes. We did not find major phenotypic differences among these individuals that could have been explained by their allele variants.

Mc and Mc+ alleles were found only in mosaic genotypes among our samples, because solid coloured dogs that could have had the heterozygous genotype were not included in this study. Therefore, it should be noted that the detected numbers of the merle allele types might not reflect their real frequency among Mudis, as we specifically tried to include the known unusually coloured merle individuals.
Correlation between genotype and health status

The health consequences of the various merle allelic sizes in homozygous genotypes are also important to mention. Thus merle to merle mating is strictly forbidden in Mudis in order to prevent the production of double merle dogs due to the conceivable health concerns in the progeny. However, not every mating takes this rule into consideration outside of the registered population, which may be attributed to either lack of knowledge or the deliberate aim to create additional, special colour variations while disregarding the possible health issues. In other breeds where breeding regulations do not restrict merle to merle mating, the presence of merle allele variants are more abundant and various risks linked to different allele combinations can be observed (Langevin et al., 2018).

Genetic testing is essential for revealing hidden and cryptic merle individuals, since both genotypes possess phenotypically invisible SINE insertions. Hidden merle dogs can carry any length of the poly-A tail, since the recessive epistatic effect of the E-locus (MC1R) does not allow the expression of the eumelanistic coat colours and the SINE insertion may be transmitted to the progeny. In the case of cryptic merle dogs, the poly-A tail is short enough, so that the splicing machinery is still able to use solely the original splice-acceptor site, resulting in normal PMEL protein, and thus colouration. However, in cryptic parent litters, puppies with the merle phenotype might also appear, due to gonadal polymorphism. During cell divisions occurring in the germ line, the poly-A tail of the SINE element can be extended in some of the germ cells. If the elongation via replication slippage reaches at least the dilute allele category, the individual will exhibit the merle phenotype (Clark et al., 2006; Kaelin and Barsh, 2013; Ballif et al., 2018; Murphy et al., 2018).

Out of the four double merle dogs involved in our study, two had severe auditory and ophthalmologic defects and only one had no noticeable ear or eye dysfunction – according to the owner’s report. However, special veterinary examinations had not been conducted focusing on these types of impairments and, therefore, partial dysfunction cannot be decisively excluded. The albino double merle had functional hearing, but had a vision deficit and photophobia, which was either caused by the merle or the albino factor respectively, or a combination of the two. The causative mutation of albinism has not been examined in the Mudi breed so far, but in other dog breeds the albino genotype has been identified as oculocutaneous albinism type 4 (OCA4) as the SLC45A2 gene mutation (Winkler et al., 2014; Wijesena and Schmutz, 2015).

Gene tests and breeding rules

DNA parentage verification of canine breeds was resolved in Hungary already in 2000 (Pádár et al., 2001), and the knowledge of extended genetic data may also be useful for well-developed breeding in order to avoid genetically-
depressed populations (Zenke et al., 2006). According to current governmental regulations in Hungary [Act 62 of 2016 (IX.16.), Ministry of Agriculture], since 1 July 2017 it has been obligatory for individuals of the Hungarian canine breeds, including Mudis, to undergo DNA parentage verification with characterised polymorphic markers (Zenke et al., 2009) in order to be officially registered. As a part of this regulation, DNA samples from every individual of the registered population will eventually be available in a central database sometime in the future. This not only provides the possibility to test the dogs for the merle locus simultaneously, but could also help to control the level of inbreeding through rational breeding and mating choices (Zenke et al., 2007, 2011). Obligatory merle testing would be beneficial for the carrier breeds due to the health concerns linked to certain genotypes, especially in those cases where the phenotype does not reveal the genotype accurately. By applying high-resolution genotyping and standardised phenotyping methods a more complex definition can be worked out on the merle phenotype with respect to the phenotype–genotype correlation. It would be advisable for cynologic associations and breed clubs to revise their breed standards and breeding rules according to recent findings, as many of them are currently not in harmony with coat-colour genetics.

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