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RESEARCH

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Investigation of the association of the *MLPH* gene with seasonal canine flank alopecia in Rhodesian Ridgeback dogs

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Abstract

Background Canine flank alopecia (CFA) is a skin condition in dogs characterized by non-inflammatory, seasonally recurring episodes of localized hair loss and often hyperpigmentation of the affected skin. A genetic basis is suspected because of the predisposition in certain breeds, such as the Rhodesian Ridgeback (RR). This study investigated the possible role of the canine *melanophylin (MLPH)* gene in CFA among RRs through pedigree analysis and *MLPH* genotyping.

Results We included 24 CFA-affected and 12 non-CFA-affected control RRs. Pedigree analysis revealed inbreeding loops and close family relationships among the CFA-affected dogs, indicating the potential heritability of CFA. *MLPH* genotyping revealed 3/24 (12.5%) affected dogs and 1/12 (8.3%) control dogs to be heterozygous (*Dd*) for the dilute (*d*) allele, indicating no difference between these groups. None of the dogs were found to be homozygous (*dd*). Statistical analysis did not reveal an association between the *MLPH-d* allele and CFA.

Conclusions The familial patterns among affected RRs observed through pedigree analysis suggest a potential genetic component in CFA. However, our findings from the *MLPH* genotyping did not reveal that the *MLPH* gene is involved in this skin condition in RRs. However, further genetic studies are needed to clarify the etiology of CFA in RR dogs.

Keywords Canine flank alopecia, Rhodesian Ridgeback, *Melanophylin* gene, Pedigree analysis, Genetic predisposition

Plain Language summary

Canine Flank Alopecia is an undesired skin condition in dogs. Affected dogs lose hair, typically on one or both sides of the body, without signs of any other skin disease. The well-demarcated bald patches are often hyperpigmented and non-itchy. The surrounding hair and skin are normal. Hair will usually regrow within 3–8 months after the onset of hair loss, but bald patches will often recur every year. Because some breeds, such as the Rhodesian Ridgeback, are at risk of developing this trait, it is suspected that this condition may have a genetic basis.

This study aimed to determine whether a specific gene (the canine *Menalophylin* gene) may play a role in canine flank alopecia among Rhodesian Ridgebacks. We used pedigree analysis to explore the relationships between family members and disease inheritance patterns within families. We used *MLPH* genotyping to examine differences in the occurrence of this gene between affected Rhodesian Ridgebacks and healthy ones. In this study, we included

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24 affected and 12 healthy control Rhodesian Ridgebacks. The pedigree analysis of the affected dogs revealed close family ties and inbreeding loops. This finding points to a possible heritability of canine flank alopecia. This genetic study did not reveal that the *MLPH* gene is involved in this skin condition. The cause of CFA may be multifactorial, with both genetic and environmental factors playing a role.

We recommend further investigation of the genetic and environmental basis of Canine Flank Alopecia in Rhodesian Ridgebacks. Deeper knowledge could help develop breeding strategies to minimize the frequency of this undesirable skin trait within the Rhodesian Ridgebacks population.

Background

Canine flank alopecia (CFA), also referred to as seasonal flank alopecia or cyclic flank alopecia, is a dermatologic condition in dogs, with a first onset between 3–6 years of age, characterized by seasonally recurring bald patches (alopecia) in the thoracolumbar region [1–4]. CFA is visually distinctive, which makes the diagnosis straightforward: non-inflammatory, non-scarring, non-pruritic, often hyperpigmented, and seasonally recurring alopecia. Benign neglect is frequently the recommended treatment of choice because affected dogs are otherwise healthy, and hair will usually regrow spontaneously within a few months [1–4]. The etiology of CFA remains unclear, although there seems to be an association with daylight exposure changes, potentially affecting the circadian rhythms and hormonal balance of dogs, especially during seasonal changes [1, 2, 4]. Some breeds (e.g., boxers, Rhodesian Ridgebacks, Airedale terriers, French bulldogs, English bulldogs, and schnauzers) are predisposed to develop CFA, suggesting that the condition is, at least in part, hereditary [1, 2, 5]. To date, studies evaluating the genetic basis of CFA are lacking.

Usually, the onset of CFA is in the months with shorter daylight. The neurohormone melatonin is influenced by daylight exposure and is known to play a role in the physiology of hair growth in various species. Given the cyclical nature of CFA and its onset during the season with declining daylight, in a previous clinical study, we investigated the efficacy of subcutaneous slow-release melatonin implants in preventing CFA recurrence in dogs.

The Rhodesian Ridgeback (RR)—a dog breed that has become popular in recent years—is predisposed to CFA [6]. Although CFA does not impair quality of life, many owners of CFA-affected dogs perceive the condition to be cosmetically undesirable. Studying the lineage of CFA-affected RRs through pedigree analysis, in conjunction with genetic testing, could offer valuable insight into the mode of inheritance and familial transmission patterns.

The c.-22G > A variant of the canine *melanophylin* (*MLPH*) gene, located on the D locus, is involved in the dilution of coat color in various breeds [7]. The *d*-allele segregates (separation of allele pairs) in the RR breed [7]. Consequently, in the RR, both *d*-allele heterozygotes and homozygotes occur. In the RR, the wheaten coat color is considered the wild type (*DD* or *Dd*, full pigmentation), while the grayish color is considered the dilute type (*dd,* dilute pigmentation). The dilute color can differ subtly from the wheaten color and is therefore not always easy to distinguish. Dilute-colored dogs may have a less pronounced color than non-homozygous dogs because of the defective transport of melanosomes in the skin. Coat color dilution in *dd MLPH*-homozygous dogs is sometimes accompanied by hair loss (coat color dilution alopecia [8] or black hair follicular dysplasia [9]).

We hypothesized that among the RR, both *MLPH-d* allele heterozygotes (*Dd)* and homozygotes (*dd)* have an increased risk of CFA compared to *MLPH-D* (*DD*) allele homozygotes. We therefore investigated familial relationships and inheritance patterns among CFA-affected RRs using a pedigree analysis. Using an *MLPH*-genotyping protocol, we investigated whether the *MLPH* gene plays a role in CFA in RRs.

Methods

Dogs

The study population consisted of purebred RRs in the Netherlands. We identified eligible CFA-affected RRs via the Rhodesian Ridgeback Club Nederland. The control group consisted of random RRs who visited the clinic for non-CFA-related conditions.

Study design

The inclusion criteria were otherwise healthy RRs with a history of at least two consecutive CFA episodes, with onset each year between November and April, followed by spontaneous full or partial hair regrowth within 8 months. We did not specifically focus on identifying dilute-colored individuals. The exclusion criterion was RRs with concurrent metabolic disease (i.e., hypothyroidism or hypercortisolism). To evaluate eligibility, owners were asked to complete a questionnaire, provide photographs of their dog with active CFA, and provide their dog's pedigree details. After inclusion, we asked the dogs' veterinary practitioner to collect EDTA-treated blood samples for DNA isolation.

Pedigree data collection

A pedigree analysis could provide clues about the mode of inheritance, in the case of monogenic inheritance. To assess the ancestry or familial relationships among affected individuals we performed a pedigree analysis to gain insights into inheritance patterns. We gathered detailed pedigree records provided by the owners of the included dogs. Within these CFA-affected dogs' pedigrees, we identified and traced common ancestors using a drawing tool to visualize familial relationships and inheritance patterns. Special attention was given to the recurrence of CFA in siblings and across multiple generations, the family relations of the parents, and the gender ratio of affected dogs. We also explored the presence of mating loops and inbreeding patterns contributing to the prevalence of CFA within familial lineages.

MLPH genotyping

For the molecular genetic study, blood samples from affected dogs were collected for DNA isolation. DNA was isolated from EDTA-treated blood samples using a MagCore® automated HF16 plus nucleic acid extractor (RBC Bioscience). A fragment containing the exon 1/intron 1 junction of *MLPH* was amplified by PCR with the forward primer sequence 5'-GTAGGACCGGAGAGAGCAG-3' and reverse primer sequence 5'-CCTGAGGCCTGTGTTTGG-3'. PCR was performed with Invitrogen Taq DNA polymerase (Thermo Fisher) at an annealing temperature of 55 °C using standard solutions and reaction times. The PCR products were treated with 1 U of exonuclease I (New England Biolabs) at 37 °C for 45 min, followed by 15 min at 75 °C. Chain termination DNA sequencing was performed with a BigDye® Terminator Kit v3.1 using reverse primers under standard conditions. The products were purified by alcohol precipitation, resuspended in 20 µl H_2O, and run on a Genetic Analyzer 3500xL (Applied Biosystems™, Thermo Fisher). The electropherograms were analyzed with Seqman Pro 14 software in the DNASTAR Lasergene package.

Sample size and statistical power

In canine genetics, approximately 10 affected and 10 control dogs are needed to achieve mapping of monogenetically inherited characteristics in the genome [10]. We determined the statistical power of the pedigree of closely related dogs for which DNA was available. A stand-alone version of Superlink was used to calculate the maximum obtainable logarithm of odds (LOD) score [11]. We used the pedigree shown in Fig. 1 and assigned homozygous and identical genotypes to the affected dogs and a heterozygous genotype to the unaffected dog A. Dogs for which no DNA was available were assigned unknown genotype (0 0). The frequency of the CFA allele was set at 0.1, and the frequency of the marker allele shared by the affected allele was also set at 0.1. We assumed recessive inheritance with full penetrance.

Results

We included 24 CFA-affected and 12 non-CFA-affected control RRs and characterized the CFA phenotype of the affected dogs. The affected dogs had a history of non-pruritic, non-inflammatory recurrent alopecic areas in the thoracolumbar region. The bilateral bald patches were sharply marginated and showed hyperpigmentation to a greater or lesser extent (Fig. 1). The skin and hair surrounding the lesions were normal, and all dogs were otherwise healthy. Spontaneous hair regrowth occurred within 3 to 8 months after CFA onset, with alopecia recurrence in subsequent years. Within the CFA group, males and females were equally often affected. The phenotypic characteristics of the study population are summarized in Table 1.

Fig. 1 Phenotypic presentation of CFA in the RR. Bilateral, sharply marginated alopecia on the flanks with lesional hyperpigmentation

Table 1 Phenotypic characteristics of the study population

Variables	Included dogs (*n* = 36)	CFA group (*n* = 24)	Control group (*n* = 12)
Age (years)			
Mean	4.6	4.8	4.3
Range	1–10	2–10	1–9
Sex			
Male	18	12	6
Female	18	12	6
Coat Color			
Wheaten	36	24	12
Number of previous CFA episodes			
Mean	n/a	2.6	n/a
Range	n/a	2–6	n/a

Pedigree analysis

We searched the pedigrees of affected dogs for common ancestors (Fig. 2). There were close family relationships between several CFA-affected dogs: one unaffected sire (A) was the father of four CFA-affected dogs (B, C, D, E) and the grandfather of two CFA-affected dogs (F, G). There was an inbreeding loop and mating loops. Sire A produced affected dogs with two sister dams. Moreover, the CFA-affected dams G and H were from the same sire. The maximum obtainable LOD score for linkage in this pedigree was 3.5, which was well above the canonical significance threshold value of 3.

MLPH genotyping

To evaluate the possible association of CFA with the *d* allele, we genotyped both CFA-affected and control dogs for the dilute locus. We found that three out of 24 (12.5%) CFA-affected dogs and one out of 12 control dogs (8.3%) were heterozygous (*Dd*) for the dilute allele, indicating that there was no significant difference between the groups. None of the dogs were found to be homozygous (*dd*) carriers of the *d* allele.

Discussion

CFA is a cosmetically undesired skin condition characterized by seasonally recurring, localized hair loss predominantly in the thoracolumbar area. Various breeds, including the RR, are more prone to develop CFA. Because of the clear breed predisposition, CFA is suspected to have a hereditary background. In this study of RRs, we investigated the familial relationships of the included CFA-affected dogs through pedigree analysis and we investigated whether the *MLPH* gene could be one of the factors that contribute to the risk of CFA in RR through *MLPH* genotyping. Among the CFA group, males and females were equally often affected, suggesting that the disorder is autosomal. The pedigree analysis of CFA-affected dogs revealed a short inbreeding loop and several mating loops, indicating that the average relatedness was greater than that of the control group. These findings show that in RRs, a genetic component appears to play a role in the etiology of CFA. The *MLPH* genotyping findings indicate that an association of CFA with the MLPH-d allele is unlikely in RRs.

There are no published previous studies on the genetic background of CFA in RRs. Some studies have been published on atypical recurrent flank alopecia (aRFA) in the Cesky Fousek [12, 13]. It should be noted, however, that there are differences in the clinical presentation between CFA and aRFA patients. Dostál et al. [12] proposed that aRFA in the Cesky Fousek is a recessive condition with incomplete penetrance. A recent genome-wide association study of aRFA in 216 Cesky Fousek dogs, using a combination of histological, genomic, and transcriptomic data, showed that the inheritance of the condition in this dog breed is multifactorial [13]. In their study, Neradilová et al. identified 4/144 candidate genes that are implicated in controlling circadian rhythm (*RORA, PIF1, TCF12, and CSNK2A1)*, which might be associated with the seasonality of aRFA. Building on these findings, further studies are warranted to investigate the potential role of these genes in the pathogenesis of CFA in RRs.

Unlike CFA in RRs, which is usually characterized by hyperpigmented, recurring bald patches primarily in the thoracolumbar area, with an onset between 3 and 6 years of age, the alopecic skin of aRFA-affected Cesky Fouseks is not hyperpigmented, and the onset often occurs later in life.

Monogenic diseases often occur at a young age, occasionally occur in littermates, and there is no sex difference. Parents of affected dogs are often closely related, and short inbreeding loops are usually present in the case of recessive inheritance.

Moreover, aRFA presents with a more extensive and diverse range of clinical manifestations: severe alopecia, affecting not only the flanks but also extending to the sacral area, thighs, and base of the tail and occasionally involving the ears and nose. These differences in the clinical presentation between CFA and aRFA may suggest that CFA in RRs has a less complex inheritance pattern than aRFA in Cesky Fousek and that the genetic basis for CFA in RRs may be monogenic.

Welle et al. [14] performed an *MLPH* c.-22G > A genotyping study combined with histopathological data in 935 dogs from 20 different dog breeds. They found that an *MLPH* mutation in various dog breeds, such as the RR, is a risk factor for the development of certain forms of hair loss (i.e., color dilution alopecia and black hair follicular dysplasia). Our *MLPH* genotyping findings did not

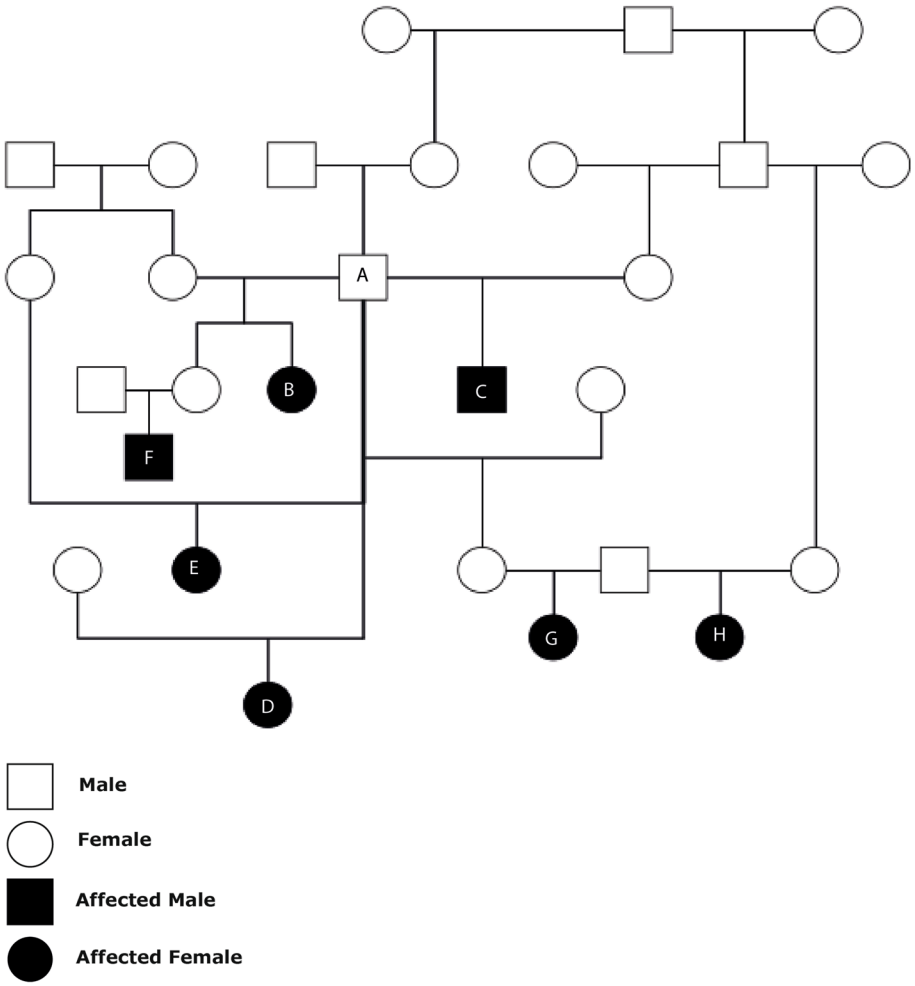

Fig. 2 Family relations of part of the study sample. DNA was available for the letter-indicated individuals

indicate an association between the dilute color allele and the development of CFA in RRs. Notably, it cannot be ruled out that another gene could be a causative factor.

To the best of our knowledge, this is the first study on the genetic background of CFA in RR dogs. Our study used a multimodal approach combining phenotypical presentation, *MLPH* genotyping, and pedigree analysis to explore the potential heritability of CFA in affected and unaffected RR dogs. Our findings are noteworthy and underline the need for further research to elucidate the etiology of CFA. In a future follow-up study, a genome-wide association study of our cohort could indicate the complexity of CFA in RRs.

The small sample size for the pedigree analysis prevented the drawing of statistically significant conclusions about inheritance patterns. Information about the CFA status in siblings of affected dogs could shed light on the mode of inheritance. To overcome this limitation in future studies, combining genetic profiling and complete litter information for pedigree analysis could strengthen the understanding of the mode of inheritance.

In conclusion, CFA in RRs is unlikely to be associated with mutations in the *MLPH* gene. Pedigree analysis, however, suggested a genetic component in the pathogenesis of CFA in RR patients. In future work, we will aim to unravel the genetic background of this undesired skin condition. Furthermore, future studies are needed to identify additional disease-promoting factors that are implicated in the development of CFA.

Abbreviations

aRFA	Atypical recurrent flank alopecia
CFA	Canine flank alopecia
LOD	Logarithm of odds
MLPH	Melanophylin
RR	Rhodesian Ridgeback

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Authors' contributions
MV: Conceptualization of the study, methodology, acquisition of the data, investigation, analysis of the data, visualization, writing the original draft of the manuscript, final approval of the manuscript version to be submitted. YS: Conceptualization of the study, supervision, revising and editing of the manuscript, final approval of the manuscript version to be submitted. PL: Conceptualization of the study, supervision, analysis of the data, validation, revising and editing of the manuscript, final approval of the manuscript version to be submitted.

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Data availability
The materials used for this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was conducted per the guidelines for Good Clinical Practice (VICH GL9). We obtained written informed consent from the participating owners, and all the data were pseudonymized.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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