Analysis of R199H Polymorphism of Canine Melanophilin Gene (MLPH) in Population of Czech Pointer in Slovakia

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Abstract

The canine melanophilin gene (MLPH) is one of three the most studies genes which is associate with coat color dilution in several breeds of dog which is characterized by a specific pigmentation phenotype. The coat color dilution is inherited as a Mendelian autosomal recessive trait and in some breeds is accompanied by hair loss which is the so-called as color dilution alopecia (CDA). The aim of this study was to analyse population of 41 dogs of breed Czech pointer for SNP polymorphism in exon 7 (G→ A transition) causing an exchange from arginine to histidine at position 199 (R199H) of the MLPH protein. Canine genomic DNA was isolated from blood by commercial kit Nucleospin Blood (Macherey Nagel). The R199H polymorphism was detected by PCR-RFLP method with restriction endonuclease *Hha*I. The allele G was detected by restriction fragments 281 bp, 193 bp, 94 bp and allele A with restriction fragments 474 bp, 94 bp. We detected all three genotypes in the population of dog breed Czech pointer. There were detected homozygote genotype GG – 32 dogs, heterozygous genotype AG – 7 dogs and recessive homozygote genotype AA – 2 dogs.

Keywords: CDA, dog, Melanophilin gene (MLPH), PCR-RFLP, polymorphism R199H.

1. Introduction

Coat color dilution in dogs is known in many breeds. This trait is inherited as a Mendelian autosomal recessive trait [1] and may be accompanied by hair loss, the so-called color dilution alopecia (CDA) [2]. The CDA is characterized by a progressive loss of hair, which is sometimes accompanied by recurrent bacterial infections of the hair follicles (folliculitis). The exposed skin of CDA affected dogs is often dry and scaly as well as sensitive to sunburn or extreme cold [3]. The pigmentation of mammalian hair and skin is a multistep process involving the synthesis of pigments (melanins) within melanosomes, the specialized, pigment-producing organelles of melanocytes and transfer the melanosomes into the hair shaft [4]. The specific pigmentation phenotype in dogs with coat color dilution is caused by defective transport of melanosomes which leads to an accumulation of melanosomes around the melanocytes' nuclei as well as large clumps of pigment in the hair shafts. Studies in the mouse identified three genes as MYO5A [5,6] RAB27A [7,8] and MLPH [4,9] expecially, which plays important role in transport of melanosomes into the hair shaft. Philipp et al. [10] mapping of the canine MLPH gene to chromosome CFA25q24. They found 48 SNPs polymorphisms in the MLPH gene but only 15 polymorphisms were in exons of which 7 led to amino acid change in the melanophilin protein. Philip et al. [3] found strong association between single nucleotide polymorphisms (SNP) localized in 7 exon of melanophilin gene (MLPH) and coat color dilution in Doberman Pinschers, Beagles, and Large Munsterlanders. The basis of this polymorphism is substition G → A in position 186184 of genomic sequence for MLPH gene (*EMBL: BN000728*). The substition G → A in the seventh exon causing a change from arginine to histidine at position 199 (R199H) of the melonophilin protein. The aim our study was to

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analyze R199H polymorphism associate with coat color dilution where is in some cases coupled with color dilution alopecia (CDA) in population of Czech pointer in Slovakia.

2. Materials and methods

In this study were collected blood samples from 43 dogs of Czech pointer breed kept in Slovakia. Canine genomic DNA was isolated by using commercial kit Nucleospin Blood (Macherey Nagel).

PCR: The PCR amplification of specific 568 bp fragment DNA included SNP in seveth exon which is characterize substitution of G/A (R199H) in position 186184 of the canine genomic *MLPH* gene sequence (*EMBL: BN000728*). For PCR amplification were used specific primers [3]. The sequence of primers were: forward primer 5´- GTC CTC AGC ACT TCT GAG - 3´ and reverse primer 5´ - GTG AGA AGC TTC TGG ACC - 3´. The reaction mixture in the total volume 25 µl containing 50 ng DNA, 1 U Taq polymerase (Fermentas), 1 x PCR buffer $(NH_4)_2SO_2$,1.5 mM $MgCl_2$, 200 µM dNTP, 10 pM of each primer. The PCR reaction was optimized in the gradient thermocycler C1000^{TM} (Biorad, USA). The following amplification parameters were applied: 95 ^oC for 3 minutes followed by 30 cycles: 95 °C for 30 seconds, 57 °C for 30 seconds, 72 °C for 45 seconds. The reaction was completed by the final synthesis: 72 °C for 10 minutes.

RFLP: The PCR products were digested by restriction endonuclease FastDigest *Hha*I (Fermentas). The digestion was performed with 10 µl of PCR product mixed with 1 µl of the restriction enzyme, 2 µl of 10 x FastDigest buffer in total volume 25 µl. The run conditions were 5 minutes at 37 ^oC. Digested fragments were visualized by electrophoresis on 2.5 % agarose gel (Invitrogen) containing GelRed dye (Biotium) at 200 V in 1 x sodium borate buffer for 15 minutes and the gel was analyzed by UV transilluminator and photographed with an documentation system Olympus C 700.

3. Results and discussion

The digestion of 568 bp PCR product with restriction endonuclease *Hha*I (Fermentas) differentiated alleles G and A. The *Hha*I digestion of the PCR products produced three fragments for allele G (281 bp, 193 bp, 94 bp) and two fragments for allele A (474 bp, 94 bp). The PCR-RFLP method was used for genotyping of 43 dogs of Czech pointer. In this group of animals were detected the all genotypes (GG, AG, AA) for single nucleotide polymorphism G/A in seveth exon of MLPH gene (Figure 1).

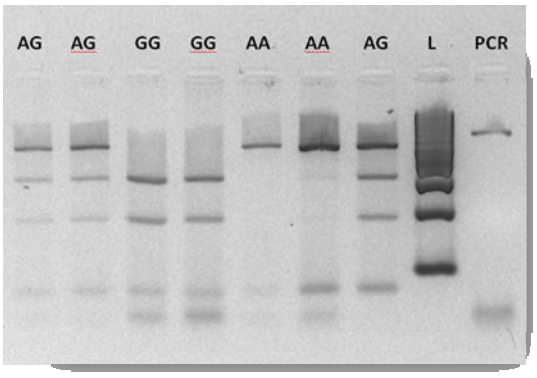

Figure 1: Representatively results of PCR-RFLP analysis for *MLPH* gene by restriction enzyme *Hha*I on 2.5 % agarose gel.
L – ladder 100 bp (Fermentas), AA genotype (474 bp, 94 bp), AG genotype (474 bp, 281 bp, 193 bp, 94 bp), GG genotype (281 bp, 193 bp, 94 bp), PCR product (568 bp).

In population of Czech pointer we detected the presence of all genotypes as homozygote genotypes GG – 32 dogs , heterozygote genotypes AG – 7 dogs and homozygote genotypes AA – 2 dogs (one dog and one female dog). The frequencies of genotypes and alleles are presented in table 1. The study detected the higher frequency of allele A (H^{199}) – 0.38 in population Doberman pinchers [3]. But they discovered the presence of genotype AA (H^{199}/ H^{199}) in four dogs of breed Large Munsterlander whereby they detected black hair follicular dysplasia (BHFD) in all tested dogs. The presence of heterozygote genotype AG was detected in one dog of breed American Staffordshire, which phenotype was dilute color and was described the occurrence of color dilution alopecia (CDA) too. The presence of homozygote genotype AA was detected in 18 dogs of German pinchers and 2 dogs of Beagles breed, where all of these dogs had a dilute phenotype. This results suggested that the polymorphism R199H can be to influence at the occurrence of dilute color in a

breeds of dogs at which in some breed as Large Munsterlander can be to cause of BHFD [3].

Table 1. Frequency of genotypes and alleles of *MLPH* gene for polymorphism R199H in the population of Czech pointer in Slovak Republic.

Czech Pointer	n	Alleles		Genotypes		
dogs female dogs	8 33	**G** (R^{199})	**A** (H^{199})	**G/G** (R^{199}/R^{199})	**A/G** (R^{199}/H^{199})	**A/A** (H^{199}/H^{199})
Total	**41**	**0.8659**	**0.1341**	**0.7805**	**0.1707**	**0.0488**

4. Conclusions

We detected the presence of all genotypes GG, AG and AA for polymorphism R199H of MLPH gene in population of Czech pointer kept in Slovakia. The homozygote genotype GG were detected in 32 dogs, heterozygote genotype AG were detected in 7 dogs and homozygote genotype AA which is associate with phenotype dilute coat were detected in 2 dogs – one dog and one female dog. The occurrence of allele A and genotype AA in population will be to lead to widespread molecular-genetic analyses because in this breed was described the presence of alopecia which can to have a genetic basis too.

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