Acta fytotechn zootechn, 19, 2016(Special Issue), 71-74
http://www.acta.fapz.uniag.sk

Short Communication

The canine melanophilin gene polymorphisms in Slovakian Rough-haired Pointer

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Article Details: Received: 2016-06-20 | Accepted: 2016-07-06 | Available online: 2016-09-01

http://dx.doi.org/10.15414/afz.2016.19.si.71-74

The aim of this study was to determine the population genotypic structure based on polymorphisms located in exon 1 (*c.-22G>A*) and exon 7 (*R199H*) of canine gene encoding melanophilin (*MLPH*). The genomic DNA were obtained from in total 49 samples of Slovakian Rough-haired Pointer and analysed using PCR-RFLP methods. The prevalence of G allele has been found in case of both identified loci. The highest frequency has been observed for heterozygous animals and its sufficient proportion was confirmed also by the coefficient of heterozygosity (0.63 or 0.67) and the F_{IS} index values (-0.29 or -0.47). The F_{ST} index showed expected high degree of genetic similarity between analysed populations resulted from their breeding purposes and common founders. The genotyping of such polymorphisms can be perspective mainly due to the fact that the *MLPH* gene was associated with the coat colour dilution and also alopecia not only in human but as well as in dog populations.

Keywords: coat colour dilution, dog, *MLPH* gene, polymorphism

1 Introduction

The domestic dogs has been bred for many purposes during the thousands of years and the creation of modern breeds started around 200 years ago. The selection of certain animals has been based mainly on few founders and breeding strategies that included strong selection criteria, popular sire or backcrossing which led to the development of disease mutations affected various phenotypic traits (Tengvall et al., 2013). The Slovakian Rough-haired Pointer (SRHP) is a recently recognized gundog breed developed after World War II in Slovakia. The breed was established by crossing German Wirehaired Pointers, Weimaraners, and the Cesky Fousek (also known as the Bohemian Wirehaired Pointing Griffon). The SRHP breed had also slight input from the German Wirehaired Pointer and Pudelpointer as well. The objective for the beginning of the SRHP breed development was to obtain individuals with great stamina which would track, point, retrieve in water or land, and be suitable for a range of prey from birds, hares and other small animals, and large game up to the size of deer. This breed was accepted by FCI (Federation Cynologique Internationale) in 1985 and categorized as a Continental Pointing Dog of the braque type. Despite its excellent hunting purposes the SRHP is currently considered as endanger mainly due to small population nucleus in Slovakia. The breed is generally fairly healthy, but several health issues have been reported for this breed. One of this that can significantly affect the coat of dogs is alopecia. Colour dilution alopecia (CDA) has been identified in Slovak and also in UK population of SRHP breed.

In many dog breeds the coat colour dilution is characterized by a specific phenotype and sometimes accompanied by hair loss and recurrent skin inflammation (so-called colour dilution alopecia or black hair follicular dysplasia) (Drögemüller et al., 2007). The CDA is defect inherited as a Mendelian autosomal recessive trait that causes poor quality of the hair coat and hair re-growth to point of progressive and extensive hair loss (Peregro et al., 2009; Lehner et al., 2013). It is relatively common in dogs with a blue or fawn dilute hair colour, mainly Blue Doberman Pinschers but also the Dachshund, Great Dane, Whippet and Poodle amongst others. However, the genetic cause of CDA is not clearly understood. It is thought that CDA is based on autosomal recessive gene transmission and

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the dilution gene may play the important role in genetic transmission of colour-mutant alopecia (Kim et al., 2005), but it is not known yet if the colour dilution gene are directly responsible for the skin changes or if a linked gene codes for the associated follicular changes. Parasites, allergy, bacterial or fungal infection, hormonal disease and imbalances of hair growth may all contribute to development of alopecia.

The mutations assigned to the genomic region of the melanophilin gene (*MLPH*) are assessed alongside *MYO5A* and *RAB26A* genes as the most important markers in relation to the coat colour dilution that can be also accompanied by alopecia. The proteins which are encoded by these genes are part of the melanosome transport complex (Phillip et al., 2005). The canine *MLPH* gene has been located in position CFA25q24 (Philip et al., 2005). Philip et al. (2005) identified within the *MLPH* gene region totally 48 single nucleotide polymorphisms (SNPs) from which only 7 led to the change in amino acid sequence of melanophilin protein. The strong associations with the coat colour dilution in dogs was found mainly for the SNPs in the exons 1, 2 and 7 (Philipp et al., 2005; Drögemüller et al., 2007; Welle et al. 2009). The causative mutation in exon 1 of *MLPH* (*c.-22G>A*) gene sequence resulting from the substitution of G→A in position 157471 is known as SNP *c.-22G>A* (Welle et al., 2009). The SNP *R199H* is a result of the G→A substitution in the exon 7 and causes amino acid change (arg→his) at position 199 in the melanophilin protein (Gábor et al., 2011a).

The aim of this study was to analyse the population genotypic structure of Slovakian Rough-haired Pointers, as small endangered dog breed kept in Slovakia, based on the identification of two polymorphisms located in the exon 1 (SNP *c.-22G>A*) and exon 7 (SNP *R199H*) of *MLPH* gene.

2 Material and methods

2.1 Biological samples and SNPs genotyping

The biological samples for analysis of genetic structure using SNPs located in canine *MLPH* gene were collected based on co-operation with two clubs of breeding organization of Slovakian Rough-haired Pointers. The genomic DNA were extracted from in total of 49 hair root samples according to protocol described by Gábor et al. (2009). The quality and concentration of genomic DNA were tested after extraction by NanoPhotometer (Implen) measuring of the optical density at wave length of 260 nm.

The analysed polymorphic sites are located within the exon 1 (*c.-22G>A*) and exon 7 (*R199H*) of the *MLPH* gene (Welle et al., 2009). The genotyping of each individual was performed using PCR-RFLP method. The PCR amplification of specific fragments were carried out by use of oligonucleotide primers designed according to Drögemüller et al. (2007) and Philipp et al. (2005) for exon 1 and exon 7, respectively. The amplification of 312 PCR fragment (SNP *c.-22G>A*) and 568 fragment (SNP *R199H*) of the *MLPH* gene was performed with appropriate reactions condition proposed by Gábor et al. (2011a). Subsequently, the products of PCR reaction were digested at 65°C and 37°C in time 10 min with 1µl of FastDigest restriction enzymes *Tsp*RI (SNP *c.-22G>A)* and *Hha*I (SNP *R199H*). Both PCR products and also restriction fragments were visualised by horizontal electrophoresis in 2% agarose gels in 0.5 x TBE (130 V for 50 min) stained with intercalate dye GelRed (Biotium).

2.2 Genetic diversity analysis

The population genotype structure and frequency of alleles were determined using the Genalex version 6.1 software (Peakall and Smouse, 2012). The same statistical environment was used for the calculation of genetic diversity indices, including observed (H_o) and expected heterozygosity (H_e), effective allele numbers (N_e), Wright's F_{IS} index indicating the molecular inbreeding coefficient and polymorphic information content (PIC). The differences between observed and expected genotype frequencies were tested by using the chi-square test in relation to the analysis of HWE in population. To infer the degree of genetic similarity between subpopulations, represented by breeding organisations that provide the samples, the F_{ST} statistic were also compute.

3. Results and discussion

The results of SNPs genotyping showed that the most frequent in case of both identified loci were the heterozygous animals. As the predominant allele in population were found the *c.-22G>A*^{G} and *R199H*^{G} alleles. The summary of the parameters reflecting the genetic diversity state in population is given in table 1. The obtained statistical significant differences ($P<0.01$) between observed and expected genotype frequencies indicated the deviations from Hardy-Weinberg equilibrium. The analysed markers showed medium level of polymorphisms and relative balanced effectiveness of alleles. The

high proportion of heterozygous animals was transferred to the high number of observed heterozygosity that confirmed also the obtained value of F_{IS} index. The Wright's F_{IS} also indicated the relative lower level of inbreeding between evaluated individuals and sufficient proportion of heterozygotes for the sustainable breeding in next generations in relation to maintainance of population biodiversity. If the population were fragmented in to the separate subpopulation based on the appropriate breeding organization the F_{ST} index showed only negligible genetic differentiation at level 0.05 that resulted mainly from the common ancestors used in the breeding history of Slovakian Rough-haired Pointers.

Table 1 The summary of genotype diversity parameters analysed in population

Locus	Genotypes			Alleles		χ^2 test	H_o	H_e	N_e	PIC	FIS
	GG	AG	AA	G	A						
c.-22G>A	0.30	0.63	0.07	0.58	0.42	8.74**	0.63	0.487	1.95	0.48	-0.29
R199H	0.31	0.67	0.02	0.64	0.36	10.67**	0.67	0.459	1.85	0.46	-0.47

**P < 0.01

The obtained higher frequency of G allele in case of both analysed SNPs of *MLPH* gene is comparable with previous published study in Czech pointer population kept in Slovakia (Gábor et al., 2011a; Gábor et al., 2011b), German Pinscher, Doberman Pinscher, Rhodesian ridgeback and for example Australian shepherd Drögemüller et al., 2007; Welle et al., 2009). But in contrast to our results, the authors found the highest proportion for homozygous GG animals that is probably responsible for dark coat colour ("wild type"). The higher proportion of heterozygous animals with dilute "A" allele in genotype can be attributed mainly to the fact that for the SRHP breed standard only Grey and Gray Roan colour variety are required.

The effect of analysed polymorphisms on coat colour dilution in dogs has been considered in several studies. Drögemüller et al. (2007) and Welle et al. (2009) reported for various dog breeds significant association between the dilute phenotype and the SNP *c.-22G>A*. Within the 20 analysed breeds Welle et al. (2007) identified for 9 populations perfect cosegregation of c.-22G>A^A allele with the dilute coat colour phenotype. In case of *R199H* polymorphisms Philipp et al. (2005) found relationship between the H^{199}/H^{199} (AA) genotype and occurrence of CDA and BHFD. Within all evaluated breeds they reported an association between diluted phenotype and the presence of R199H^A allele in animal's genotype.

3 Conclusions

The genotypic structure determined based on both *c.-22G>A* and *R199H* polymorphisms across analysed population of Slovakian Rough-haired pointers is comparable with previously published studies of various breeds. The prevalence of heterozygous in population has been attributed mainly to the SRHP breeding purposes. The obtained sufficient proportion of genetic variability for the future sustainable breed management in relation to maintain of population biodiversity was found. The genotyping of polymorphisms in the *MLPH* gene in SRHP population can be perspective in the future not only for evaluation of diversity but also for explanation its association with the coat colour dilution and also alopecia. Despite that the genetic cause of CDA is not clearly clarified future studies can help us to understand the relationship between colour dilution and *MLPH* gene. This test can help determine carriers of the dilution gene and eliminate it from populations, in which it is undesirable.

Acknowledgments

This work has been supported by the Slovak Research and Development Agency (Contract No. APVV-14-0054).

References

DRÖGEMÜLLER, C. et al. (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. *Journal of heredity*, vol. 98, no. 5, pp. 468–473. doi: http://dx.doi.org/10.1093/jhered/esm021

GÁBOR, M. (2009) *Genetic markers of meat quality in cattle and sheep*: Dissertation thesis. Nitra: SPU (in Slovak).

GÁBOR, M. et al. (2011a) Genetic polymorphims of MLPH gene in Czech pointer breed. In: *International Conference BALNIMALCON 2011 „Improvement and diversification of Balkan animal production within the European context*“, 19 - 21. October 2011, Bucharest – Romania.

GÁBOR, M. et al. (2011b) Analysis of R199H Polymorphism of Canine Melanophilin Gene (MLPH) in Population of Czech Pointer in Slovakia. *Scientific Papers: Animal Science and Biotechnologies*, vol. 44, no. 1, pp. 213–215.

KIM, J. H. et al. (2005) Color-dilution alopecia in dogs. *Journal of Veterinary Science*, vol. 6, no. 3, pp. 259–261.

LEHNER, S. et al. (2013) Two-exon skipping within MLPH is associated with coat color dilution in rabbits. *PloS One*, vol. 8, no. 12, e84525. doi: http://dx.doi.org/10.1371/journal.pone.0084525

PEAKALL, R. and SMOUSE, P. E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, vol. 6, pp. 288–295. doi: http://dx.doi.org/10.1111/j.1471-8286.2005.01155.x

PEREGO, R. et al. (2009) Color dilution alopecia in a blue Doberman pinscher crossbreed. *Canadian Veterinary Journal*, vol. 50, pp. 511–514.

PHILIPP, U. et al. (2005) Polymorphisms within the canine MLPH gene are associated with dilute coat color in dogs. *BMC Genetics*, vol. 16, no. 6, pp. 34. doi: http://dx.doi.org/10.1186/1471-2156-6-34

TENGVALL, K. et al. (2013) Genome-wide analysis in German shepherd dogs reveals association of a locus on CFA 27 with atopic dermatitis. *PloS Genetics*, vol. 9, no. 5, e1003475. doi: http://dx.doi.org/10.1371/journal.pgen.1005740

WELLE, M. et al. (2009) *MLPH* Genotype—Melanin Phenotype Correlation in Dilute Dogs. *Journal of heredity*, vol. 100 (Supplement 1), pp. 75-79. doi: http://dx.doi.org/10.1093/jhered/esp010