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Short communication

Prevalence of the *SOD1*, *PRCD* and *SLC2A9* gene mutations responsible for degenerative myelopathy, progressive rod-cone degeneration, and hyperuricosuria in Polish population of Labrador Retriever dogs

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Abstract

Knowledge of the molecular background of hereditary diseases facilitates the unambiguous diagnosis of affected animals and the identification of healthy carriers, which is particularly important from a breeding perspective. To date 330 canine diseases with at least one known causative variant have been described. Degenerative myelopathy (DM), caused by a mutation in the *SOD1* gene; progressive rod-cone degeneration (PRCD), caused by a mutation in the *PRCD* gene; and hyperuricosuria (HUU), caused by a mutation in the *SLC2A9* gene, are among the most common monogenic autosomal recessive diseases identified in numerous dog breeds; however, their incidence varies significantly among breeds. The Labrador Retriever is a popular breed in Poland, and it was assumed that the known causative DNA variants for these three diseases are also present in its gene pool. The aim of this study was to analyze the distribution of these causal mutations in the Polish population of this breed. In total, 200 dogs were studied using Sanger sequencing. Among them, 32 carriers (16%) and 4 affected individuals (2%) were identified for PRCD, and 2 carriers (1%) were identified for HUU, while all studied dogs were free of the *SOD1* mutation. The results obtained were compared with data for over 16,800 Labrador Retrievers published by Donner et al. (2023). We concluded that the frequency of the causal mutation responsible for DM in the Polish population is lower, while the frequencies of the causative variants for PRCD (0.01) and HUU (0.005) are slightly higher.

Keywords: dog, canine, Labrador Retriever, monogenic disease, *PRCD*, *SCL2A9*, *SOD1*

Introduction

Presently, the Online Mendelian Inheritance in Animals (OMIA, https://www.omia.org/home/) database includes 330 canine hereditary diseases with at least one known causal genetic variant. To date, more than thirty monogenic diseases, mostly inherited in an autosomal recessive manner, have been reported in Labrador Retrievers. Among them, degenerative myelopathy (DM), progressive rod-cone degeneration (PRCD), and hyperuricosuria (HUU) are common in many breeds; however, DM is relatively rare in Labrador Retrievers (Donner et al. 2018, 2023). Degenerative myelopathy is characterized by progressive degeneration of the spinal cord, leading to paraplegia and tetraparesis. It is caused by a G>A transition in exon 2 of the *SOD1* gene (rs853026434, p.Glu40Lys) (Awano et al. 2009, OMIA:000263-9615). Progressive rod-cone degeneration, a common inherited retinal disease, is caused by a C>T transition in exon 1 of the *PRCD* gene (rs852451717, p.Cys2Tyr) (Zangerl et al. 2006, OMIA:001298-9615). Hyperuricosuria, which can result in urate urolithiasis and the formation of kidney and bladder stones (Bartges and Callens 2015), is caused by a G>T transversion in exon 5 of the *SLC2A9* gene (rs1152388406, p.Cys188Phe) (Bannasch et al. 2008, OMIA:001033-9615).

According to the 2022 breeding report from the Polish Kennel Club, Labardor Retriever is a popular breed in Poland, with 837 registered females and 336 registered stud dogs (https://www.statista.com/statistics/1400974/poland-breeding-retrievers-flushers-and-water-dogs-by-sex/).

We hypothesized that the above mentioned causal DNA variants are also present in the gene pool of the Polish population of Labrador Retrievers, although their distribution remains unknown. The aim of this study was to estimate for the first time the frequency of these three causal mutations in Polish Labradors.

Material and Methods

Genomic DNA previously isolated from blood samples collected from a random cohort of unrelated Labrador Retriever dogs, with the approval of the Local Ethical Commission for Investigations on Animals in Poznań (31/2013; May 17, 2013) for studies on the molecular background of canine obesity (National Science Centre, grant no. 2013/09/B/NZ2/02208), was used in the present study. A total of 200 DNA samples were used for standard PCR amplification. Primers for the *PRCD* (F: CATTCCCTGACACATGCAAC and R: GGGAAACCTCTCTGGACCTC; amplicon size 596 bp) and *SLC2A9* (F: TTCTTGTCAGAGCACGGCAG and R: GTGAAGGACCTGAGTGCTCA; amplicon size 427 bp) variants were designed using Primer3Plus software (version 3.3.0) and the annealing temperature was established at 60ºC. The primers for the *SOD1* (F: GTCCCCAGCCTAGAATGGTTAA and R: CGGCTTTGTGGATCATTTCC; amplicon size 438 bp) variant had annealing temperature at 53°C and were adopted from Turba et al. (2017). DNA sequencing was performed on a 3500 Series Genetic Analyzer (Applied Biosystems HITACHI) using 3500 Data Collection Software v3 (Applied Biosystems) and the obtained sequences were aligned to reference gene sequences (NC_051835.1 for *SOD1*, NC_051813.1 for *PRCD*, and NC_051807.1 for *SLC2A9*) using DNASTAR Navigator software (version 17.5.0.48). Genetic equilibrium was assessed, and the *PRCD* and *SLC2A9* variants were found to be in Hardy-Weinberg equilibrium (HWE).

Results and Discussion

All 200 dogs were homozygous for the wild-type variant (GG) at the *SOD1* locus. The amplified *SOD1* PCR fragment also overlapped several other known SNP sites (rs3350794591, rs851394212, rs3350566504, rs3351803741, rs3352384058, rs3350566395, rs3349190769, rs3351803618, rs3551608519, rs851171028, rs3350566490, rs3351358437, and rs3351825614); however, all of these loci were monomorphic for the reference variant in the analyzed cohort. The absence of the causal *SOD1* variant aligns with the findings of Donner et al. (2023), who analyzed 16,855 Labrador Retrievers and reported extremely low frequencies of the pathogenic A variant (0.0058) and the homozygous AA genotype (0.0000336) (Table 1).

Analysis of the *PRCD* gene revealed 4 dogs (2%) homozygous for the pathogenic variant (TT), 32 dogs (16%) were carriers (CT), and 164 dogs (82%) were homozygous for the wild-type variant (CC). The distribution of genotypes in the studied cohort confirmed to Hardy-Weinberg equilibrium. The frequency of the pathogenic T allele and the TT genotype were slightly lower than those reported by Donner et al. (2023) in Labrador Retrievers (Table 1). Unfortunately, we were not able to monitor the health status of the dogs with the causative homozygous recessive genotype. The amplified fragment also overlapped with several other known SNP sites (rs333218583, rs3333562688, rs850632775, rs3333523933, rs3333608580, rs3333528367, rs3333591523, rs852754241, and rs3333250494). Among these, only one SNP-rs852754241 (9:4864948G>A) located in the 5′-flanking region –

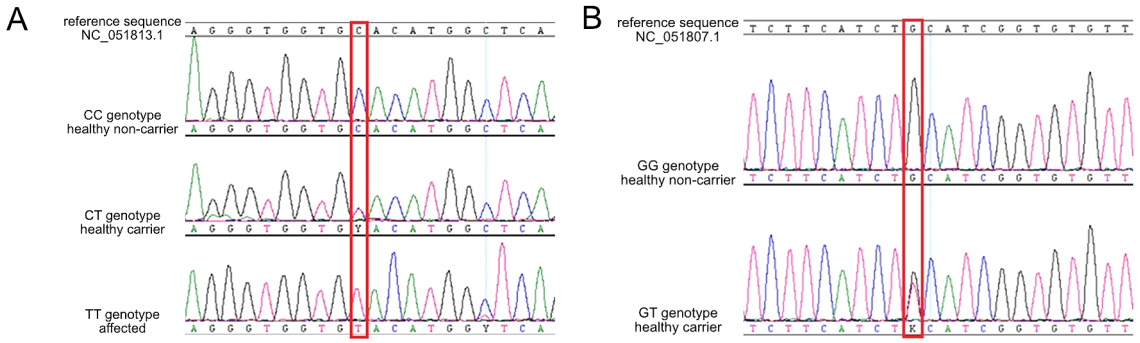

Fig. 1. Chromatograms presenting genotypes of the studied dogs at: A) the g.4188663C>T SNP (rs852451717) in PRCD, and B) the g.69456869G>T SNP (rs1152388406) in SLC2A9. Rectangles indicate the positions of the SNPs.

Table 1. Comparison of the DNA variants and genotypes frequencies for *SOD1, PRCD* and *SCL2A9* genes in Labardor retriver dogs.

Disease (Gene)	Panel of purebred dogs DM, N=242 641 PRCD, N=242 217 HUU, N=242 658 (Donner et al. 2023)					Panel of labrador retrievers DM, N=16 855 PRCD, N=16 825 HUU, N=16 856 (Donner et al. 2023)					Present study N=200				
	Allele frequencies		Genotype frequencies			Allele frequencies		Genotype frequencies			Allele frequencies		Genotype frequencies		
	G	A	G/G	G/A	A/A	G	A	G/G	G/A	A/A	G	A	G/G	G/A	A/A
Degenerative myelopathy – DM (*SOD1*)	0.9096	0.0904	0.8437	0.1318	0.0245	0.9942	0.0058	0.9884	0.0115	0.00003	1.00	0.00	1.00	0.00	0.00
	C	**T**	**C/C**	**C/T**	**T/T**	**C**	**T**	**C/C**	**C/T**	**T/T**	**C**	**T**	**C/C**	**C/T**	**T/T**
Progressive Rod-Cone Degeneration – PRCD (*PRCD*)	0.9839	0.0161	0.9698	0.0282	0.0020	0.9281	0.0719	0.8614	0.1334	0.0052	0.9	0.1	0.82	0.16	0.02
	G	**T**	**G/G**	**G/T**	**T/T**	**G**	**T**	**G/G**	**G/T**	**T/T**	**G**	**T**	**G/G**	**G/T**	**T/T**
Hyperuricosuria - HUU (*SCL2A9*)	0.9802	0.0198	0.9653	0.0298	0.0049	0.9993	0.0007	0.9986	0.001399	0.0000005	0.995	0.005	0.99	0.01	0.00

was polymorphic in the studied population. For this SNP, variant frequencies, calculated for 153 genotyped dogs, were 0.698 (G) and 0.302 (A).

The causative T variant in the *SLC2A9* gene was identified in only two heterozygous dogs (1%), resulting in a very low frequency of this variant (0.005) (Table 1). The distribution of genotypes in this cohort was in Hardy-Weinberg equilibrium. Interestingly, the frequency of the pathogenic variant in the cohort studied by Donner et al. (2023) was even lower (q=0.0007). Within the amplified PCR fragment, a known SNP site (G>T, rs9148103) was also detected. The variant frequencies for this SNP in 179 genotyped dogs were 0.978 for the G variant and 0.022 for the T variant. Additionally, a known insertion/deletion variant (rs9148104, intronic variant c.594+12_594+13insC) was detected. The frequencies of the delC and insC variants, analyzed in 200 dogs, were 0.985 and 0.015, respectively. Two other known SNP sites (rs3327540385, rs852993612) that overlapped with the amplified fragment were monomorphic.

Degenerative myelopathy (DM), diagnosed in over 120 dog breeds (Donner et al. 2018) with the highest frequency in German Shepherds and Chesapeake Bay Retrievers (Mandrioli et al. 2020, Santos et al. 2020), affects older dogs (age > 8 years) homozygous for the A variant in the *SOD1* gene (Awano et al. 2009, Donner et al. 2018). However, it is important to note that some AA homozygotes may not exhibit DM symptoms, highlighting that the disease is age-related and exhibits incomplete penetrance. It is worth noting that canine DM serves as a model for human amyotrophic lateral sclerosis (ALS) (Awano et al. 2009).

Progressive rod-cone degeneration (PRCD) has been identified in more than 50 dog breeds (Donner et al. 2023), with the disease being most frequently diagnosed in Labrador Retrievers and Toy Poodles, according to a global study on the canine population (Clark et al. 2023). PRCD in dogs is also considered a model for human retinitis pigmentosa (RP), as it displays similar clinical symptoms and is caused by mutations in the orthologous *PRCD* gene. Interestingly, the causative mutation identified in dogs (C>T transition leading to a cysteine-to-tyrosine amino acid substitu-

tion, rs852451717) has also been diagnosed in a woman with RP, with the corresponding human variant being rs121918369 (Zangerl et al., 2006).

Hyperuricosuria (HUU) has been diagnosed in over 30 dog breeds, with the highest incidence observed in Dalmatians, Russian Black Terriers, and English Bulldogs (Karmi et al., 2010). In humans, nephrolithiasis has also been associated with mutations in the *SLC2A9* gene (Matsuo et al. 2008).

Conclusions

Our study has shown that the DNA variant responsible for degenerative myelopathy (DM) is either absent or present at very low frequency in the gene pool of the Polish Labrador Retriever population. This aligns with previously published data, which report a very low incidence of this causative variant in the global population of the breed. In contrast, the incidence of the mutation responsible for progressive rod-cone degeneration (PRCD) is relatively high, while the incidence of the variant causing hyperuricosuria (HUU) is low. However, for both diseases, the observed incidence was higher in the Polish population than in the global population.

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