Acta fytotechn zootechn, 19, 2016(Special Issue), 111-113
http://www.acta.fapz.uniag.sk

Short Communication

A genetic study of a SOD1 missense mutation in Czechoslovakian Wolfdog

Daniela Jakabová[1*], Patrícia Chlebovcová[1], Martin Genčík[1]

Vetgene/Medgene s.r.o., Bratislava, Slovak Republic

Article Details: Received: 2016-06-23 | Accepted: 2016-07-26 | Available online: 2016-09-01

http://dx.doi.org/10.15414/afz.2016.19.si.111-113

Canine degenerative myelopathy occurs in many dog breeds, mainly German Shepherd dogs. The causative mutation E40K in the SOD1 gene changes the structure and function of the superoxide dismutase 1 protein. The genetic makeup of the Czechoslovakian Wolfdog breed, that resulted from hybridization between Carpathian wolves and German Shepherds in the 1950s, shows a higher proportion of the dog than wolf genetic background. In the present study we have screened the Czechoslovakian Wolfdog population for the causative degenerative myelopathy mutation E40K in order to estimate its prevalence in Slovakia. Our study confirmed that the frequency of the mutant allele A (0.25) is relatively high and genetic testing is important in breeding programs.

Keywords: dogs, SOD1 gene, DM

1 Introduction

Canine degenerative myelopathy (DM) is a progressive and devastating neurodegenerative disease causing progressive paralysis. Dogs with DM show an insidiously progressive ataxia and paresis of the pelvic limbs (Capucchio et al., 2014). All affected dogs show clinical signs of DM by the age of 8 years (Holder et al., 2014). DM is an autosomal-recessive genetic disease caused by the c.118G>A (E40K) mutation in the SOD1 gene (Awano et al., 2009).

The Czechoslovakian Wolfdog is an unique dog breed, that resulted from hybridization between Carpathian wolves and German Shepherds (Smetanová et al., 2015). Its genetic composition shows a higher proportion of the dog genome than wolf genome. The aim of our study was to genotype causative mutation E40K in the Czechoslovakian Wolfdog population in Slovakia.

2 Material and methods

Blood samples were originated from 54 Slovak Czechoslovakian Wolfdogs. DNA was extracted from 300 µl blood using Wizard Genomic DNA purification Kit (Promega). PCR reactions were performed in 10 µl volume consisting of 1µl of extracted DNA (~ 40ng), 0.5 mM of each primer, 1x Thermo-Start PCR Master Mix (Thermo Scientific) following PCR amplification protocol 15 min. at 95 °C, 30 s. at 95 °C, 30 s. at 59 °C and 1.30 min. at 72 °C for 35 cycles and final extension 10 min. at 72 °C. Primer sequences were designed according to the reference sequence NM_001003035.1 from NCBI database. The PCR fragments were cycle sequenced using the BigDye Terminator Cycle sequencing Kit version 1.1 and were run on an Avant 3100 Genetic Analyser (Applied Biosystems). The resulting sequences were aligned and compared with Geneious software (Biomatters).

3 Results and discussion

Genetic studies in familial canine degenerative myelopathy detected the association between the disease and mutation in the superoxide dismutase 1 (SOD1) gene (Holder et al.,2014). In 2009, Awano and colleagues reported that dogs with histopathologically confirmed DM were homozygous for

* **Corresponding Author:** Daniela Jakabová, VetGene / Medgene s.r.o., Ondrejovova 30, 821 03 Bratislava, Slovak Republic. E-mail: daniela.jakabova@vetgene.eu

the A allele of a SOD1 gene in exon 2 causes an E40K missense mutation. The disease was originally described in the German Shepherd dog, but homozygosity for the A allele was strongly associated with DM in over 115 breeds.

In our study, a total of 54 individuals of Czechoslovakian Wolfdogs breed from Slovakia were genotyped for the causal mutation E40K in the SOD1 gene: 23 individuals (0.375) were heterozygotes A/G, 29 dogs were homozygotes G/G (0.5625) for the ancestral allele G and 2 dogs were homozygous A/A (0.0625) for the mutant A allele (Figure 1).

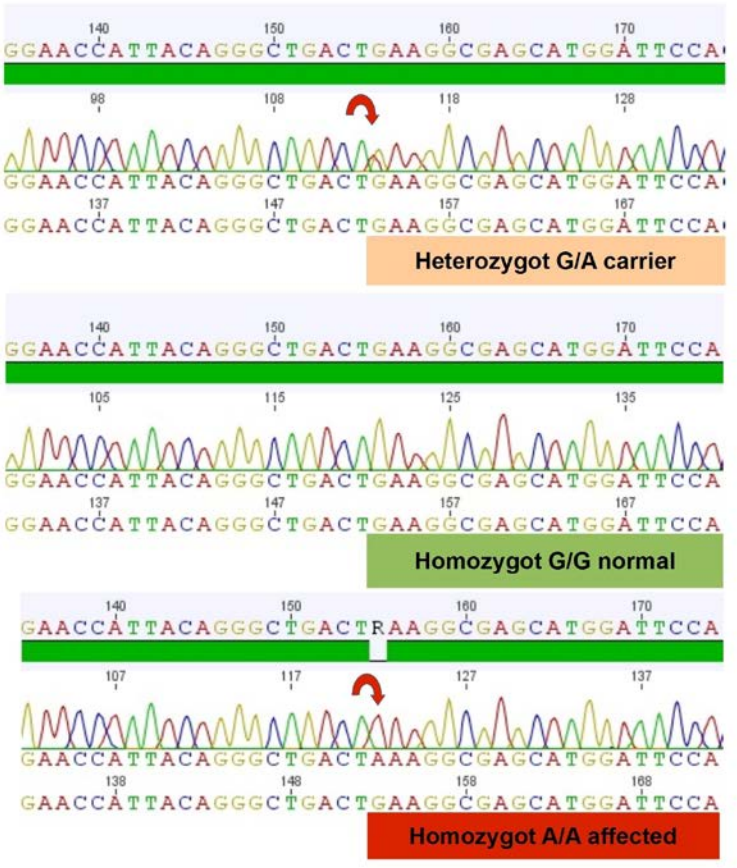

Figure 1 The genetic screening for the mutant allele SOD1:c.118G>A.

The frequency of the allele A was estimated on level 0.25 in the Slovak population. Previously, a higher frequency of mutant allele A was detected in American study of Czechoslovakian Wolfdogs (0.34) where from 52 individuals 7 were mutant homozygous, 21 carriers and 24 wild-type homozygous (Zeng et al., 2014).

Our study suggests that genotyping for the mutant A allele may be useful component of the diagnostic panel and would help to reduce the prevalence of the causative mutation in the Czechoslovakian Wolfdog population over several generations.

4 Conclusions

In the present study of Czechoslovakian Wolfdogs we confirmed the high occurrence of heterozygotes (0.375) and essential role of genetic screening for the mutant allele SOD1:c.118G>A in breeding programs. Genetic identification of carriers and their exclusion from reproduction can help to reduce the spreading of mutant allele and decrease the risk of DM in populations with limited numbers of individuals such as in Czechoslovakian Wolfdogs in Slovakia.

References

AWANO, T. et al. (2009) Genome-wide association analysis reveals a SOD1 mutation in canine degenerative myelopathy that resembles amyotrophic lateral sclerosis*. Proceedings of the National Academy of Sciences USA*, vol.106, pp.2794–2799.

CAPUCCHIO, M.T. et al. (2014) Degenerative myelopathy in German Shepherd Dog: comparison of two molecular assays for the identification of the SOD1:c.118G > A mutation*. Molecular Biology Reproduction*, vol. 41, pp.665–670.

HOLDER, A. L. et al. (2014) A retrospective study of the prevalence of the canine degenerative myelopathy associated superoxide dismutase 1 mutation (SOD1:c.118G > A) in a referral population of German Shepherd dogs from the UK. *Canine Genetics and Epidemiology*, vol.1, pp.10.

SMETANOVÁ, M. et al. (2015) From Wolves to Dogs, and Back: Genetic Composition of the Czechoslovakian Wolfdog. *PLoS ONE*, vol.10, pp.12. doi: http://dx.doi.org/10.1371/journal.pone.0143807

ZENG, R. et al. (2014) Breed Distribution of SOD1 Alleles Previously Associated with Canine Degenerative Myelopathy. *Journal of Veterinary Internal Medicine*,vol. 28, pp. 515–521.