Frequency of canine degenerative myelopathy SOD1:c.118G>A mutation in 22 dog breeds in Guadalajara, Mexico¤

Frecuencia de la mutación SOD1:c.118G>A de mielopatía degenerativa canina en 22 razas de perros en Guadalajara, México

Frequência da mutação SOD1: c.118G>A de mielopatía degenerativa canina em 22 raças de cães no Guadalajara, México

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Abstract

Background: Canine degenerative myelopathy (DM) is a late-onset disease that primarily affects large-breed dogs. The disease involves the spinal cord and produces progressive paresis and, eventually, complete loss of mobility. DM has been related to missense mutation c.118G>A in the *SOD1* gene. **Objective:** To determine the genotypic and genic frequencies of DM in Mexico. **Methods:** In total, 330 samples from 22 different dog breeds were genotyped using the polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP) technique. **Results:** The mutation was identified in 71 animals from 11 different breeds. Observed genic frequencies were 0.78 for the G allele and 0.14 for the A allele. Genotypic frequencies were 0.79 for the G/G wild-type, 0.14 for the G/A heterozygote, and 0.7 for the A/A homozygote. **Conclusion:** The genic frequency of this allele is high among the studied populations. A molecular marker program that identifies the DM mutation in breeding dogs should be implemented in order to reduce this frequency.

Keywords: *genetic disease, molecular marker, PCR-RFLP, SOD1 gene.*

Resumen

Antecedentes: La mielopatía degenerativa canina (MD) es una enfermedad progresiva de presentación tardía que afecta a la médula espinal, generalmente en caninos de razas grandes, y que produce paresis progresiva y eventual pérdida completa de la movilidad. Se ha relacionado con una mutación puntual por

sustitución de bases en el gen *SOD1* recientemente identificado como c.118G>A. **Objetivo:** Determinar las frecuencias genotípicas y génicas para la presentación de DM en México. **Métodos:** Se genotipificaron 330 muestras de perros de 22 razas mediante la técnica de reacción en cadena de la polimerasa y polimorfismos de longitud de fragmentos de restricción (PCR- RFLPs). **Resultados:** Se identificó la mutación en 71 animales de 11 razas diferentes. Las frecuencias génicas encontradas fueron de 0,78 para el alelo G y de 0,14 para el alelo A. Las frecuencias genotípicas fueron de 0,79 para el tipo silvestre G/G, 0,14 para el heterocigoto G/A y 0,7 para el homocigoto A/A. **Conclusión:** La frecuencia encontrada para la mutación es alta en las poblaciones estudiadas. La aplicación de un programa de selección asistida por marcadores moleculares contra la mutación causante de MDC en perros reproductores resultaría útil para reducir su frecuencia.

Palabras clave: *enfermedad genética, gen SOD1, marcador molecular, PCR-RFLPs.*

Resumo

 Antecedentes: A mielopatía degenerativa canina (MD) é uma doença progressiva de apresentação tardia que afeta a medula espinal geralmente de caninos de raças grandes e que produz paresia progressiva e eventualmente a perda completa da mobilidade. Tem sido relacionada com uma mutação pontual por substituição de bases no gen *SOD1*, recentemente identificado como c.118G>A. **Objetivo:** Determinar as frequências genotípicas e genéticas para a apresentação de DM no México. **Métodos:** Genotipagem de 330 amostras de cães de 22 raças por meio da técnica de reação em cadeia da polimerase e polimorfismos no comprimento de fragmentos de restrição (PCR- RFLPs). **Resultados:** A mutação foi identificada em 71 animais de 11 raças diferentes. As frequências gênicas encontradas foram de 0,78 para o alelo G e de 0,14 para o alelo A. As frequências genotípicas foram de 0,79 para o tipo silvestre G/G, 0,14 para o heterozigoto G/A e 0,7 para o homozigoto A/A. **Conclusão:** A frequência encontrada para a mutação é alta nas populações estudadas. A implementação de um programa de seleção assistida por marcadores moleculares contra a mutação que causa MDC seria útil para reduzir a sua frequência.

Palavras-chave: *doença genética, gen SOD1, marcador molecular, PCR-RFLPs.*

Introduction

Canine degenerative myelopathy (DM) is a late-onset neurodegenerative disease generally reported in large-breed dogs that affects the white matter of the spinal cord (March *et al.,* 2009). DM slowly degenerates the upper motor neurons of the pelvic limbs, causing progressive paresis and proprioceptive ataxia (Miller *et al.,* 2009). Complete loss of mobility is observed 6 to 12 months after the first signs appeared (Cappucchio *et al.,* 2014). Thoracic-limb functionality as well as fecal and urinary continence usually remain unaffected until the terminal stage of the disease. Although genetic, metabolic, nutritional, vascular, and immune-mediated etiologies have been proposed, a specific pathogenic process remains to be identified (March *et al.,* 2009).

DM was originally considered to affect only the German Shepherd breed (Awano *et al.,* 2009). However, the pathology has since been identified in approximately 125 different dog breeds (Zeng *et al.,* 2014). A definitive diagnosis for DM can only be confirmed by a postmortem spinal cord histopathologic study (Cappucchio *et al.,* 2014). DM-associated degenerative damage includes axonal loss and demyelization of the spinal cord, mainly in the caudal thoracic area (Nakamae *et al.,* 2015). Variations in the nervous lesions described for the disease exist between different breeds and even within a single breed. Nonetheless, the degenerative changes to white matter funiculi observed in all breeds involve dilation of the myelin sheath, axonal swelling, as well as fragmentation and phagocytosis of myelin and axonal debris (March *et al.,* 2009).

DM appears to be an autosomal recessive, incompletely penetrant disease related to a missense mutation in the superoxide dismutase 1 (*SOD1*) gene (Capucchio *et al.,* 2014), which can be identified by PCR. A 2009 report found that dogs with a confirmed postmortem histopathological DM diagnosis were homozygous for the A allele in missense mutation *SOD1*:c.118G>A, which predicts a p.E40K amino acid substitution in the *SOD1* gene (Zeng *et al.,* 2014).

The identification of the DM causing mutation could be useful to differentiate this disease from other progressive thoraco-lumbar syndrome causing illnesses, such as intervertebral disk disease and diskospondylitis (Jones *et al.,* 2005). The objective of the present study was to determine the genotypic and genic frequencies for DM in Guadalajara, Mexico, using the polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP) technique.

Materials and methods

Ethical considerations

This study was approved by the Bioethics Internal Regulation Committee of the Academic Centre of Biological and Agricultural Sciences, University of Guadalajara, Mexico (CC/NN11-12/00/2012).

Study samples

A total of 330 blood samples were obtained from 22 dog breeds and associated crossbreeds. Breeds were selected taking in account previous reports which had identified them as DM-susceptible breeds. Sampled breeds were: Australian Cattledog, Australian Shepherd, Belgian Malinois, Border Collie, Boxer, Cocker Spaniel, Collie, Dalmatian, English Bulldog, French Bulldog, German Shepherd, and crossbreeds, Golden Retriever, Kerry Blue Terrier, Labrador Retriever, Old English Sheepdog, Pembroke Welsh Corgi, Poodle, Pug, Rhodesian Ridgeback, Rottweiler, and crossbreeds, Shetland Sheepdog, and Siberian Husky. Blood samples were stored in EDTA tubes and refrigerated before processing. Some samples were submitted through the Small Animal Clinic of the Veterinary Medicine and Animal Science School of the University of Guadalajara. Others were donated to the University of Guadalajara by dog owners or their veterinarians.

Sample genotyping

We extracted DNA from blood samples using the Quick DNA™ Universal Kit (Zymo Research, Orange, CA, USA). For genotyping through PCR-RFLP, we used the Thermo Scientific™ DreamTaq™ PCR kit (Takara Bio, Inc., Kusatsu, Shiga, JAP) with 20 µL of reaction mix containing ~100 ng of blood lysate DNA, 2 µL of 1X PCR buffer containing 20 mM $MgCl_2$, 1 µL of 10 mM dNTP mix, 0.5 µL of DreamTaq DNA Polymerase, 1.25 pMol of both primers, and the remaining volume of double-distilled water (ddH_2O). Samples were then digested with the AcuI restriction endonuclease (New England Biolabs, Inc., Ipswich, MA, USA) in a Techne® TC-5000 (Techne Inc., Burlington, NJ, USA). Primers used to identify the gene encoding *SOD1* were those described by Awano *et al.* (2009; GenBank ID: NM_001003035).

Amplification was conducted in a Techne® TC-5000 (Techne Inc., Burlington, NJ, USA) thermal cycler with the following PCR program: Initial denaturing for 5 min at 95 °C, with 35 consecutive cycles consisting of denaturing at 94 °C for 20 s, alignment at 50 °C for 30 s, extension at 72 °C for 30 s, and final extension at 72 °C for 5 min. We analyzed amplification products by 4% agarose gel electrophoresis with GelRed™ (Biotium, Hayward, CA). Results were visualized under ultraviolet light.

Results

A total of 330 dogs from 22 breeds and their crossbreeds were analyzed and genotyped to identify the *SOD1* gene mutation associated with DM. Out of all analyzed animals, 24 representing four breeds were identified as homozygous for the mutation (AA), corresponding to 7.27% of the entire sample; 47 animals from 11 breeds were identified as heterozygous for the mutation (AG), corresponding to 14.24%. The resulting frequency for the A allele was 0.14. Table 1 shows the A allele distribution, according to breed. The most representative breed for this study was the Pembroke Welsh Corgi, since it exhibits the highest A allele frequency despite occupying the fifth place in number of sampled animals per breed.

Discussion

A total of 330 samples from 22 dog breeds were analyzed. Exactly half of these breeds carried the studied mutation. Zeng *et al.* (2014) published a

Table 1. A allele distribution in 11 the breeds with the c.118G>A mutation.

Breed	N	G/G	(%)	G/A	(%)	A/A	(%)	q
Border Collie	80	79	(99)	1	(1)	0	(0)	0.01
Boxer	62	34	(55)	21	(34)	7	(11)	0.28
Poodle	33	29	(88)	4	(12)	0	(0)	0.06
German Shepherd	31	25	(81)	4	(13)	2	(6)	0.13
Pembroke Welsh Corgi	21	0	(0)	7	(33)	14	(67)	0.83
Belgian Malinois	18	14	(78)	3	(17)	1	(6)	0.14
English Bulldog	12	10	(83)	2	(17)	0	(0)	0.08
Old English Sheepdog	9	8	(89)	1	(11)	0	(0)	0.06
Rottweiler	7	6	(86)	1	(14)	0	(0)	0.07
Pug	3	2	(67)	1	(33)	0	(0)	0.17
Australian Shepherd	2	0	(0)	2	(100)	0	(0)	0.50

n: Number of dogs tested; G/G: Two normal alleles; G/A: Heterozygous; A/A: two mutant alleles; q: Mutant allele frequency; %: Percent of dogs per category.

retrospective study about the allelic distribution of the *SOD1* gene in various dog breeds, where the total A allele frequency was estimated to be 0.37. In our study, the total frequency was 0.14, which reinforces the assertion that the *SOD1*:c.118G>A mutation is widespread across different dog populations. Similarities exist between the high A allele frequencies reported by various authors for the Pembroke Welsh Corgi breed. For example, Zeng *et al.* (2014) reported a frequency of 0.79, and Awano *et al.* (2009) reported a frequency of 0.70. Both values are similar to that obtained in our study (0.83). These results demonstrate that, in the aforementioned breed, the *SOD1* mutation is present in various dog populations, regardless of geographic distancing. These findings also state DM is not restricted to a specific geographic zone.

Capucchio *et al.* (2014) found a total A allele frequency of 0.17 in the German Shepherd. The authors referred to this figure as a high and risky frequency for a mutant allele. Many of the frequencies found in our study were similar (German Shepherd 0.13, Belgian Malinois 0.14, Boxer 0.28), demonstrating that these frequencies represent an important factor to consider in the constant manifestation of DM. Both Pembroke Welsh Corgi and Boxer breeds display an elevated A allele frequency, while also showing the highest number of homozygotic animals. In a study conducted by Awano *et al.* (2009), seven DM-affected dogs were identified through histopathology, all of which had an A/A genotype. This finding suggests that there is an extremely high risk for the A/A homozygotic animals to eventually exhibit DM clinical signs.

In conclusion, we were able to identify the *SOD1* mutation associated with DM in 11 dog breeds through PCR-RFLP. The *SOD1*:c.118G>A mutation is present in various dog breeds in Mexico, in some cases with particularly high frequency. A molecular marker that could be used to assist with breeding selection would allow breeders to reduce this genetic factor influencing DM incidence, while helping to diminish its presentation in future dog generations.

Acknowledgments

Conflicts of interest

The authors declare that they have no conflicts of interest with regard to the work presented in this report.

References

Awano T, Johnson GS, Wade CM, Katz ML, Johnson GC, Taylor JF, March PA. Genome-wide association analysis reveals a SOD1 mutation in canine degenerative myelopathy that resembles amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 2009; 106(8):2794-2799.

Capucchio MT, Spalenza V, Biasibetti E, Bottero MT, Rasero R, Dalmasso A, Sacchi P. Degenerative myelopathy in German Shepherd Dog: Comparison of two molecular assays for the identification of the SOD1: c. 118G> A mutation. Mol Biol Rep 2014; 41(2):665-670.

Jones JC, Inzana KD, Rossmeisl JH, Bergman RL, Wells T, Butler K. CT myelography of the thoraco-lumbar spine in 8 dogs with degenerative myelopathy. J Vet Med Sci 2005; 6(4):341-348.

March PA, Coates JR, Abyad RJ, Williams DA, O'Brien DP, Olby NJ, Oglesbee M. Degenerative myelopathy in 18 Pembroke Welsh corgi dogs. Vet Pathol 2009; 46(2):241-250.

Miller AD, Barber R, Porter BF, Peters RM, Kent M, Platt SR, Schatzberg SJ. Degenerative myelopathy in two Boxer dogs. Vet Pathol 2009; 46(4):684-687.

Nakamae S, Kobatake Y, Suzuki R, Tsukui T, Kato S, Yamato O, Kamishina H. Accumulation and aggregate formation of mutant superoxide dismutase 1 in canine degenerative myelopathy. Neuroscience 2015; 303:229-240.

Zeng R, Coates JR, Johnson GC, Hansen L, Awano T, Kolicheski A, Guo J. Breed distribution of SOD1 alleles previously associated with canine degenerative myelopathy. J Vet Intern Med 2014; 28(2):515-521.