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 paresia 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 paresia progresiva y eventual pérdida completa de la movilidad. Se ha relacionado con una mutación puntual por


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stomatization of bases in the gene SOD1 recently identified as c.118G>A. **Objective:** Determine the frequencies genotypic and genomic for the presentation of DM in Mexico. **Methods:** Genotyped 330 dogs of 22 different breeds with the technique of reaction in chain of polymerase and polymorphisms of restriction fragments (PCR-RFLPs). **Results:** Identified the mutation in 71 animals of 11 different breeds. The genotypic frequencies were 0.78 for the homozygous G/G, 0.14 for the heterozygous A/A and 0.7 for the homozygous A/A. **Conclusion:** A frequency of mutation is high in the populations studied. The implementation of a program of selection assisted by molecular markers for the mutation that causes MDC would be useful to reduce its frequency.

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

**Summary**

**Background:** Canine degenerative myelopathy (MD) is a progressive 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).

**Conclusion:** A frequency of mutation is high in the populations studied. The implementation of a program of selection assisted by molecular markers for the mutation that causes MDC would be useful to reduce its frequency.

**Keywords:** genetic disease, SOD1 gene, molecular marker, 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 into 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 DNATM 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 MgCl₂, 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 (ddH₂O). 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
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Table 1. A allele distribution in 11 the breeds with the c.118G>A mutation.

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

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.

A 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

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Conflicts of interest

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


