Detection of a genetic mutation for myotonia congenita among Miniature Schnauzers and identification of a common carrier ancestor

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Objective—To develop a molecular genetic test to detect the mutant skeletal muscle chloride channel (ClC-1) allele that causes myotonia congenita in Miniature Schnauzers and to analyze the relationship of affected and carrier dogs.

Animals—372 Miniature Schnauzers from the United States, Canada, Australia, and Europe that were tested between March 2000 and October 2001.

Procedure—The sequence surrounding the mutation in the ClC-1 allele was amplified by use of a unique pair of primers. Polymerase chain reaction (PCR) products were digested with the restriction enzyme Hpy CH4 III and separated on a 6% polyacrylamide gel. Pedigrees from all available carrier and affected dogs were analyzed, and a composite pedigree was established.

Results—Enzyme digestion of PCR products of the normal ClC-1 allele resulted in 3 fragments of 175, 135, and 30 bp, whereas PCR products of the mutant allele resulted in fragments of only 175 and 165 bp. Of the 372 Miniature Schnauzers, 292 (78.5%) were normal, 76 (20.4%) were carriers, and 4 (1.1%) were affected (myotonic) dogs. Frequency of the mutant allele was 0.113. Pedigree analysis revealed that a population ancestor of all carriers and affected dogs.

Conclusions and Clinical Relevance—A PCR-based enzyme digestion DNA test was developed. The objective was to develop a specific DNA test to help limit the spread of this deleterious mutation. (Am J Vet Res 2002;63:1443–1447)

Myotonia congenita is a hereditary pathogenic condition affecting skeletal muscle ion channels and is characterized by a delay of relaxation of the skeletal muscles following an electrical or mechanical stimulus or after cessation of voluntary activity. Myotonia congenita results from genetic defects in the skeletal muscle chloride ion channel (ClC-1) and the ensuing reduced chloride ion conductance across the sarcolemma. Myotonia congenita has been described in mice, sheep, goats, horses, cats, and dogs, including Chow Chows, Staffordshire Bull Terriers, Great Danes, and Miniature Schnauzers.

Myotonia congenita has been characterized best in Miniature Schnauzers, a breed in which it is inherited as an autosomal recessive trait. However, the frequency of this disease in Miniature Schnauzers is unknown. Myotonic Miniature Schnauzers have hypertrophic skeletal muscles, difficulty in rising after a period of rest, a stiff and stilted gait when walking, and a bunny-hop type movement when running. In addition, there are increased respiratory sounds, difficulty when swallowing, ptalmial, dental abnormalities, and superior prognathism, a unique feature of myotonia congenita in this breed. The biochemical defect relates to reduced chloride conductance across the skeletal muscle membrane resulting in increased sarcolemmal excitability.

The molecular defect is a missense mutation resulting in a C to T transition in the ClC-1 allele predicting the replacement of a threonine residue (ACG codon) by methionine (ATG codon) in the amino terminal of the ClC-1 gene. On the basis of sequence alignments, this amino acid residue corresponds to threonine-268 in the ClC-1 allele of humans.

The objectives of the study reported here were to develop a restriction enzyme-based DNA test for the detection of the mutant gene responsible for myotonia congenita in Miniature Schnauzers, determine the frequency of this mutant allele among tested dogs, and conduct a pedigree analysis for affected (myotonic) and carrier Miniature Schnauzers.

Materials and Methods

Animals—Dogs used in the study were from breeding kennels or were privately owned dogs in the United States, Canada, Europe, and Australia. Dogs were solicited for the study through announcements made by the Miniature Schnauzer Club of America and Canada and also through posting of information about the study on our university Web site. Most of the dogs were from the United States and Canada.

Blood samples were collected from Miniature Schnauzers into EDTA-containing tubes, or buccal swab specimens were obtained with small brushes. Supported in part by the Josephine Deubler Genetic Disease Testing Laboratory at the School of Veterinary Medicine, University of Pennsylvania, and the National Institutes of Health (RR 02512). Presented in part at the American College of Veterinary Internal Medicine Forum, Denver, Colo, May 23, 2001. Drs. John Melniczek, Susan Volk, and Al George for technical assistance.

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Extraction and amplification of genomic DNA—
Genomic DNA was extracted from EDTA-anticoagulated blood by use of a commercial kit used in accordance with the manufacturer's protocol. Brushes containing buccal swab specimens were processed for DNA isolation by use of a DNA isolation kit.

Polymerase chain reaction (PCR) assay—The sequence of genomic DNA surrounding the site of the disease-causing mutation in the CIC-1 allele in Miniature Schnauzers was amplified by use of a flanking forward primer (intron 6, with 21 bases of the sequence 5'-GTT GTC CCC ATC TTC AGC CCC–3') and a reverse primer (ie, 733-714R; located in exon 7, with 20 bases of the sequence 5'-A CTC CCA CAG CAC AGC CCA C–3'). The PCR cycling conditions included an initial denaturation step at 94°C for 3 minutes. Three cycles were performed; each cycle consisted of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. This was followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. Final extension was at 72°C for 3 minutes. Each 50-µL reaction mixture contained 38.15 µL of deionized water, 0.3 µL of the forward primer (40 µM), 0.3 µL of the reverse primer (40 µM), 4 µL of 2.5 mM deoxynucleoside triphosphate mixture, 5 µL of 10X PCR buffer that contained 15 mM MgCl₂, 0.25 µL of Taq DNA polymerase (5 U/µL), and 2 µL (approx 100 ng) of genomic DNA.

Restriction enzyme digestion and gel electrophoresis—The PCR products were subjected to digestion with the restriction enzyme Hpy CH4 III. Digestion was conducted at 37°C for 2 hours in a final volume of 10 µL with 5 µL of deionized water, 3 µL of PCR product, 1 µL of enzyme, and 1 µL of New England buffer 4X. The digested PCR products were separated on a 6% polyacrylamide gel by use of electrophoresis.

Results
DNA test procedure—Optimal PCR and enzyme digestion conditions were established to reproducibly amplify the DNA segment of interest and identify the mutant and normal CIC-1 allele. After Hpy digestion, PCR products of the normal allele were separated electrophoretically on a 6% polyacrylamide gel and cut into 3 fragments of 175, 135, and 30 bp, whereas the mutant allele was cut into only 2 fragments of 175 and 165 bp (Fig 1 and 2). These results were consistent with size estimates determined on the basis of results for the molecular-weight ladder. Samples from carrier dogs revealed all 4 DNA fragments, as expected. An invariant Hpy recognition site within the PCR product served as an internal-control product for enzyme digestion, producing a 175-bp fragment in all samples. Samples from Miniature Schnauzers that were known to be normal, carrier, and affected (myotonic) dogs, as determined by use of planned matings and another molecular method used to identify the mutant allele, were blank sample, and a 100-bp ladder served as internal-control samples for each assay, which contained up to 10 samples. Primer pairs were species-specific (normal-sized PCR products were amplified from other breeds of dogs) and did not amplify DNA from humans or produce other-sized DNA products when DNA from other species such as domestic cats was used (data not shown).

Mutation screening—We tested 367 EDTA-anticoagulated blood samples and 5 buccal swab samples by use of the PCR-based DNA test for myotonia congenita in Miniature Schnauzers. Of the 372 samples tested, 292 (78.5%) were homozygous normal dogs, 76 (20.4%) were heterozygous carriers, and 4 (1.1%) were homozygous affected dogs (Table 1). These data indicated a mutant allele frequency of 0.113. Reasons owners opted to have DNA testing for their Miniature Schnauzers were intention to breed (118 dogs; 31.7%), participation in general genetic screening within the breed (181; 48.7%), relatives known to be affected or carriers (66; 17.7%), or dog had clinical signs that made the owners suspect it was affected by the condition (7; 1.9%).

Of the 7 dogs in the last group, 4 had clinical evi-
ence of myotonia and also were homozygous for the mutant allele, whereas a review of the clinical signs of the remaining 3 dogs, which did not have the mutant allele, indicated that they did not have typical clinical signs of the condition. Of the 4 homozygous myotonic Miniature Schnauzers, 3 were from eastern Pennsylvania and 1 was from Ohio, and all of them had typical signs of myopathy and superior prognathism. The DNA test results of the 7 dogs with clinical signs were confirmed by analysis of DNA sequencing, which revealed only the mutant allele in the 4 affected dogs, whereas the other 3 dogs had the normal sequence for the ClC-1 allele (data not shown).

Carrier dogs were found throughout the United States, Canada, Australia, and Scandinavia. Of the 372 dogs tested, 277 were privately owned, whereas 95 dogs were from 22 breeding kennels. Of all the dogs tested, 117 (31.9%) were champions, including 105 from the United States, 9 from Canada, and 3 from Australia. There were 10 (13.3%) champion dogs among the 76 carriers, including 3 male and 7 female dogs. Of the 372 Miniature Schnauzers tested, 143 were males and 197 were females; sex was not reported for 32 dogs. The male-to-female ratio was 1:1.38 for normal dogs, whereas it was 1:2 for carrier dogs. At the time of testing, dogs ranged from 1 month to 11 years of age.

Table 1—Geographic distribution and test results for the mutation of the chloride channel allele responsible for myotonia congenita in Miniature Schnauzers

<table>
<thead>
<tr>
<th>Location</th>
<th>Total</th>
<th>Carriers</th>
<th>Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pennsylvania</td>
<td>61</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>California</td>
<td>48</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Michigan</td>
<td>27</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Illinois</td>
<td>20</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Florida</td>
<td>24</td>
<td>3</td>
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</tr>
<tr>
<td>Wisconsin</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Indiana</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maryland</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ohio</td>
<td>11</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Other states with &lt; 10 samples</td>
<td>72</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Total for United States</td>
<td>307</td>
<td>64</td>
<td>4</td>
</tr>
<tr>
<td>Ontario</td>
<td>42</td>
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</tr>
<tr>
<td>Yukon</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Saskatchewan</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Quebec</td>
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<td>0</td>
</tr>
<tr>
<td>Total for Canada</td>
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</tr>
<tr>
<td>Switzerland</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Australia</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Denmark</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sweden</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total for other countries</td>
<td>9</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>372</td>
<td>76</td>
<td>4</td>
</tr>
</tbody>
</table>

Pedigree analysis—Pedigree information was received from the owners or breeders of 62 of 76 carriers and 3 of 4 affected Miniature Schnauzers. Pedigree analysis identified a common ancestor and popular sire that was a carrier, as determined by results of the DNA test. A composite pedigree consisting of 42 carrier and 3 affected dogs revealed that the common carrier ancestor was responsible for the genetic transmission of myotonia congenita in Miniature Schnauzers (Fig 3). Pedigree information was not available for the fourth myotonic Miniature Schnauzer. Furthermore, the relationship of the additional 34 carriers and the common carrier ancestor was too complex to be documented in the pedigree figure.

Figure 3—Composite pedigree of 43 carrier and 3 affected Miniature Schnauzers that were tested by use of a DNA-based test for a mutation that would result in myotonia congenita. A common ancestor (arrow) was identified and found responsible for genetic transmission of myotonia congenita in this breed. Other affected Miniature Schnauzers for which pedigree information was available were also related to that same common ancestor but are not included in this composite. Black square = Affected (myotonic) male dog. Black circle = Affected (myotonic) female dog. Open square = Untested male. Open circle = Untested female. Open square containing a black circle = Carrier male. Open circle containing a black circle = Carrier female.
Discussion

Myotonia congenita is 1 of nearly 400 hereditary diseases described in dogs. A diagnosis of myotonia congenita is suggested in puppies as young as a few weeks of age on the basis of typical clinical features such as myotonia and skeletal muscle hypertrophy, and it can be confirmed by characteristic electromyographic discharges. In contrast to the condition in other breeds, myotonia congenita in Miniature Schnauzers also is associated with severe prognathism and is caused at the molecular level by a missense mutation in the CIC-1 allele.

In the original description of the molecular defect in myotonic Miniature Schnauzers, separate allele-specific hybridization assays with radioactive probes were used to identify the mutant and normal alleles, which required special equipment and did not include internal-control specimens for each sample. Control dogs that were heterozygous and homozygous for the mutation in that study also are descendants of the common heterozygous ancestor tested and identified in the study reported here. We designed a test with a set of primers and enzyme digestion suitable for screening of Miniature Schnauzers for this mutation. Subjecting the PCR product to digestion with the restriction enzyme Hpy and subsequent polyacrylamide gel separation of PCR products allowed for unequivocal differentiation between normal and mutant alleles and provided an internal-control specimen as a result of an invariant cutting of the normal and mutant alleles by Hpy. This DNA test is accurate and can be performed on buccal swab specimens or EDTA-anticoagulated blood samples obtained from Miniature Schnauzers, but it probably would not be useful in other breeds with myotonia congenita, because different CIC-1 mutations may result in the same clinical condition. Affected dogs of other breeds were not available for testing.

In humans, mutations in the CIC-1 gene have been reported in isolated families from various parts of the world. Twenty-two mutations cause the autosomal recessive form of myotonia congenita (Becker type), and 7 mutations cause the dominant form (Thompson type). However, none of them had the same mutation as the myotonic Miniature Schnauzers. Frequency surveys of specific CIC-1 mutations in human populations have not been conducted.

All 4 myotonic Miniature Schnauzers were homozygous for the deleterious mutation and had clinical signs typical of the condition. These dogs are being medically managed with procainamide. The other 3 dogs that were tested because of clinical signs that made their owners suspicious that the dogs were affected did not have the CIC-1 mutation and did not have clinical myotonia. Thus, the DNA test proved clinically useful in helping to confirm or rule out myotonia congenita in Miniature Schnauzers with clinical signs of myotonia.

Only a few myotonic Miniature Schnauzers have been identified thus far. Because of their serious disabilities, these dogs are not used for breeding. However, the mutant allele can be spread through the breeding of carriers. Thus, identification of carrier dogs is crucial in the control of this myopathy. Whereas clinically normal parents of myotonic dogs must be obligate carriers, other carriers cannot be discovered except by DNA testing or unnecessary and ethically questionable test matings. The mutation-specific DNA test described here offers an opportunity to identify all 3 genotypes (normal, carrier, and affected) in Miniature Schnauzers, thereby allowing informed planned matings without the production of myotonic puppies and further spread of the mutant alleles.

For this initial survey of 372 Miniature Schnauzers that was conducted from March 2000 until October 2001, an astounding 20.4% were carriers and 1.1% were affected dogs. Carriers were identified in the United States and Canada as well as in Australia, Denmark, and Sweden, suggesting a world-wide distribution that may be attributable to the popularity of the carrier ancestor and his offspring. It is not surprising that more females than males were tested, because substantially more females are used for breeding than are males. Interestingly, all carriers and myotonic dogs for which pedigree information was available were related to 1 common ancestor, which we determined was a carrier. That dog was a champion and popular for use as a sire. We do not have evidence that carriers for the myotonia mutation would be phenotypically superior to other Miniature Schnauzers, but it is possible that carriers may be slightly better muscled and have a more pronounced gait than normal Miniature Schnauzers and, thereby, may be preferred by judges at dog shows. However, champion Miniature Schnauzers did not have a larger proportion of carriers than was evident among the other dogs. Similarly, bovine leukocyte adhesion deficiency, in which a common ancestor bull was determined to be a carrier, does not provide an advantage for performance related to carrier status.

Although myotonic Miniature Schnauzers described in other studies also were related to the common carrier ancestor reported in this study, it remains unknown whether that dog was truly the first Miniature Schnauzer to carry this mutation. The true origin of a mutation for a recessive disorder is rarely recognized in dogs or other species, but this information would be helpful in determining the spread and control of the disease. For instance, the mutation for X-chromosomal severe combined immunodeficiency in Cardigan Welsh Corgis could be traced back through only a few generations and was completely eradicated by spaying all carrier bitches.

Each year, approximately 30,000 Miniature Schnauzers are registered with the American Kennel Club, but only a small and biased group of dogs have been screened for this mutation. As information about the common carrier ancestor spread and owners provided additional information, it was found that many of the dogs tested were related to that ancestor. Although the Hardy-Weinberg equilibrium may not be appropriate to use in view of random mating and biased samples, the predicted values for normal, carrier, and affected dogs were 292.7, 74.5, and 4.7, respectively. However, the observed mutant allele frequency of 0.113 is likely much higher than expected in the general population of Miniature Schnauzers, because a disproportionate number of dogs related to the carrier ancestor were tested.
Similarly, high frequencies of mutant alleles have been reported for a few other diseases in various breeds of dogs for which a DNA-based test is available, including 0.16 for cystinuria in Newfoundland dogs,0.03 for fucosidosis in English Springer Spaniels,8 0.13 for type-III von Willebrand disease in Scottish Terriers,27 0.02 for progressive retinal atrophy in Irish Setters,26 0.325 for copper toxicosis in Bedlington Terriers,27 and 0.112 and 0.065 for leukocyte adhesion deficiency in Irish Setters26 and Red and White Setters.27 respectively. However, all those studies involved examination of a limited number of dogs (ranging from 50 to approximately 1,000 subjects), and the studies were biased, because the samples were submitted by interested parties. In comparative surveys, investigators found the frequency for a mutant phosphofructokinase allele was 0.014 in a randomized study of champion English Springer Spaniels, whereas it was 0.135 for a biased longitudinal study of English Springer Spaniels. The frequencies were consistent with the predicted distribution that was determined on the basis of the Hardy-Weinberg equation.

Miniature Schnauzers that are intended for breeding whose parents have not been documented to have normal alleles should be screened for the CIC-1 mutation. If only dogs with normal alleles are used for breeding, or if carrier dogs are mated only with dogs that have normal alleles as determined on the basis of DNA testing, this myopathy can be successfully eliminated from Miniature Schnauzers within 1 or 2 generations.

References