Identification of microsatellite markers linked to progressive retinal atrophy in American Eskimo Dogs

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Objective—To identify microsatellite markers linked to progressive retinal atrophy (PRA) in American Eskimo Dogs.

Sample Population—Blood samples or buccal epithelial cells from 66 American Eskimo Dogs, including 53 PRA-unaffected and 13 PRA-affected dogs.

Procedure—The genotypes of unaffected and affected dogs were determined by use of microsatellite markers spanning canine chromosome 9 (CFA09). Homozygosity mapping was used to detect linkage between markers and the gene locus for PRA.

Results—Significant allelic association between marker alleles and the gene locus for PRA was detected for GALK1 and TK1, indicating linkage between these markers and the causative gene locus for PRA.

Conclusions and Clinical Relevance—These data indicate that PRA in American Eskimo Dogs is located on CFA09 and allow for the development of a microsatellite-based test to identify carrier (unaffected) and affected dogs before clinical signs appear. (Am J Vet Res 2005;66:1900–1902)

Progressive retinal atrophy (PRA) is a heterogeneous group of hereditary retinal diseases leading to blindness and occurring in more than 100 breeds of the domestic dog, Canis familiaris.1 Included among these is the American Eskimo Dog. Initial signs include the loss of nighttime vision, followed by the progressive loss of daytime vision and complete blindness.1 Rate of progression and age of onset are variable among breeds.1 Early and late forms of disease onset exist. In late-onset PRA, clinical signs appear in adolescence or adulthood but appear prior to this time in early-onset PRA.3 The American Eskimo Dog is affected with a late-onset form, with signs first occurring around 3 to 6 years of age. Progressive retinal atrophy and its various forms are not restricted to dogs. A phenotypically similar disease, retinitis pigmentosa, exists in humans. Understanding the genetics underlying PRA in dogs may provide insight into human studies of retinitis pigmentosa.

Transmission of PRA can be through autosomal recessive, autosomal dominant, or X-linked mechanisms. Autosomal recessive forms of PRA include progressive rod-cone degeneration, rod-cone dysplasia 1, rod-cone dysplasia 2, rod dysplasia, photoreceptor dysplasia, and early retinal degeneration.2 Interestingly, some forms of the disease are breed specific.1 However, progressive rod-cone degeneration, a late-onset autosomal recessive form of PRA, has been identified in at least 8 breeds and has been mapped to the centromeric region of canine chromosome 9 (CFA09) near the GALK1 and TK1 loci.1 To date, a causative mutation for progressive rod-cone degeneration has not been characterized, although several candidate genes have been excluded.

In an effort to identify chromosomal regions harboring genes that may be causative for PRA, researchers have performed whole genome screens to identify microsatellite markers that cosegregate with the disease.3 Microsatellite markers are tandem repeats of 1 to 6 bp that are evenly dispersed throughout the genome and are useful in mapping disease traits as a result of their Mendelian inheritance and highly polymorphic nature. During replication, polymorphisms arise from polymerase errors, resulting in the addition or removal of tandem repeats. When a microsatellite marker is located in close proximity to a disease allele, the 2 loci are coinherited or linked. Identification of a marker linked to a disease allele allows for the development of a marker-based test to identify carrier and affected dogs. A more specific and accurate test is a mutation-based test in which the causative mutation is detected.3 Mutations causative for and markers linked with various forms of PRA have been identified in several breeds; however, no mutation or linkage has been identified in American Eskimo Dogs.

The best available tool for whole genome screens of dogs is the minimal screening set 2 (MSS-2), a set of 327 canine microsatellite markers that provides 9-Mb coverage of the canine genome.5 The MSS-2 has been multiplexed into 69 chromosome-specific panels and provides an efficient tool for whole genome scans.7 Use of the multiplexed MSS-2 reduces the time and expenses necessary to conduct linkage studies.

Determination that a particular microsatellite marker segregates with a disease can be accomplished through linkage disequilibrium (LD) analysis. This method requires a young population with certain structures, including genetic isolation, a small number of founders, expansion by growth, and rare disease alleles.5 On the basis of these criteria, purebred dogs are well suited for LD mapping. Most modern dog breeds have been developed within the last 300 years from a small
number of founding ancestors. Each breed is a genetically isolated subpopulation with limited gene flow as a result of the pedigree barrier. On the basis of the unique genetic structure of purebred dogs, LD mapping assumes that the mutation causative for a disease occurred as a founder event and that all or most affected individuals in subsequent generations inherited the disease alleles and associated markers from this common ancestral origin. Over time, recombination events occur and LD decays, so only markers that are very close to the disease locus remain in allelic disequilibrium.

Our goal in the study reported here was to identify a marker for PRA that could be used for early diagnosis and identification of carrier dogs to help reduce the incidence of disease alleles in American Eskimo Dogs. We used homozygosity mapping (ie, LD mapping of recessively inherited traits) in this study to detect linkage of PRA in American Eskimo Dogs to microsatellite markers on CFA09.

**Materials and Methods**

**Protocol**—Blood or buccal epithelial cell samples were collected from 66 American Eskimo Dogs, and genomic DNA was isolated by use of a commercial DNA isolation kit. Stocks of DNA were maintained at a concentration of 50 ng/µL. Dogs included in the study were examined by board-certified veterinary ophthalmologists to determine phenotypic status. On the basis of information obtained from the Canine Eye Registration Foundation on relatives of the dogs examined, partial pedigrees of the American Eskimo Dogs were assembled for analysis.

The 11 multiplexed microsatellite markers in the MSS-2 and an additional 2 markers spanning CFA09 were chosen for amplification and analysis (Table 1). The 11 multiplexed microsatellite markers were amplified in 2 reactions, and the 2 additional markers were amplified individually. The forward primer of each pair was labeled with 1 of 4 fluorescent dyes (6-carboxy-fluorescein and 3 other commercially available dyes). Markers were amplified as described by Clark et al.

Genotypes were collected from 66 dogs, of which 53 were unaffected and 13 were affected. Polymerase chain reaction products were resolved with an internal size standard. The 13 aforementioned markers were used; of these, 12 were reliably amplified. The distribution of affected and unaffected groups were evaluated by use of the Fisher exact test for 2 × 2 tables. For each marker, the allele present in the homozygous state and more often associated with affected dogs was chosen, and all other alleles were grouped into a single class. Under the null hypothesis, the marker of interest is in complete Hardy-Weinberg equilibrium and linkage equilibrium with the gene locus for PRA. The accepted P value for significance in LD analysis is P < 0.0001. In the study reported here, therefore, a value of P < 0.0001 results in rejection of the null hypothesis and provides evidence for LD and thus linkage between the marker and gene locus for PRA.

**Results**

To determine the mode of inheritance of PRA in American Eskimo Dogs, partial pedigrees were assembled. Analysis of these partial pedigrees revealed that unaffected offspring were produced from the mating of a PRA-affected dog with an unaffected dog. Equal numbers of affected males and females were seen, and several affected dogs were produced from unaffected parents. Taken together, these data suggest that PRA in American Eskimo Dogs results from a single, fully penetrant autosomal recessive mutation.

The 13 aforementioned markers were used; of these, 12 were reliably amplified. The distribution of alleles and map locations of microsatellite markers on CFA09 were determined (Table 1). Genotypes for all affected dogs included alleles at the GALK1 locus and two 122-bp alleles at the TK1 locus. Genotypes for all affected dogs included alleles at the MYL4 locus and two 122-bp alleles at the TK1 locus. Genotypes for all affected dogs included alleles at the MYL4 locus and two 122-bp alleles at the TK1 locus. Genotypes for all affected dogs included alleles at the TK1 locus.

**Table 1.—Alleles and canine chromosome 9 map locations of microsatellite markers.**

<table>
<thead>
<tr>
<th>Microsatellite markers</th>
<th>Intermarker distances (Mb)</th>
<th>No. of alleles</th>
<th>Sizes of alleles (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GALK1</td>
<td>1.0</td>
<td>6</td>
<td>175, 177, 181, 183, 185, 187</td>
</tr>
<tr>
<td>TK1</td>
<td>4.8</td>
<td>3</td>
<td>118, 120, 122</td>
</tr>
<tr>
<td>MYL4</td>
<td>5.7</td>
<td>1</td>
<td>104</td>
</tr>
<tr>
<td>FH2263</td>
<td>9.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C09.173</td>
<td>13.6</td>
<td>5</td>
<td>99, 101, 103, 105, 107</td>
</tr>
<tr>
<td>REN54L20</td>
<td>21.1</td>
<td>4</td>
<td>147, 149, 151, 153</td>
</tr>
<tr>
<td>GO6401</td>
<td>26.7</td>
<td>2</td>
<td>125, 133</td>
</tr>
<tr>
<td>FH2186</td>
<td>35.9</td>
<td>12</td>
<td>356, 472, 476, 480, 488, 492, 496, 520, 524, 540, 544, 548</td>
</tr>
<tr>
<td>REN145P07</td>
<td>42.8</td>
<td>5</td>
<td>208, 216, 218, 220, 222</td>
</tr>
<tr>
<td>REN367E35</td>
<td>48.2</td>
<td>4</td>
<td>248, 252, 256, 260</td>
</tr>
<tr>
<td>REN32K24</td>
<td>64.4</td>
<td>5</td>
<td>196, 198, 200, 204, 206</td>
</tr>
<tr>
<td>FH2985</td>
<td>73.6</td>
<td>9</td>
<td>188, 190, 194, 196, 200, 202, 204, 206, 214</td>
</tr>
<tr>
<td>REN287G01</td>
<td>77.1</td>
<td>4</td>
<td>183, 185, 187, 211</td>
</tr>
</tbody>
</table>

*Mb = Megabases. ND = Not determined.*
position of the gene locus for progressive retinal atrophy.

Concerns have been raised about the frequency of obtaining false-positive results when related individuals are used in LD mapping.15 Because some of the dogs included in the analysis were related, we addressed this by recalculating P values with the inclusion of only those dogs that were known to be unrelated by at least 3 generations. This resulted in 15 unaffected and 6 affected dogs. A P value of significance was still obtained for GALK1 (P < 0.0001), thus validating our conclusions.

Discussion

Autosomal recessive, autosomal dominant, and X-linked forms of PRA have been reported7 for dogs. On the basis of examination of partial pedigrees, autosomal dominant and X-linked forms of PRA can be eliminated as possible modes of inheritance in American Eskimo Dogs. It seems most likely that autosomal recessive is the mode of inheritance for this disease, as is true for several other breeds.7

The most prevalent autosomal recessive late-onset form of PRA is progressive rod-cone degeneration, which has been mapped to CFA09.1 This information, along with the knowledge that PRA in American Eskimo Dogs has a late onset, revealed that investigation of an autosomal recessive disease would be an obvious starting place. Therefore, in our study, 13 markers on CFA09 were chosen for amplification and analysis, and all were reliably amplified with the exception of FH2263.

P values were not calculated for MYL4, REN73K24, and FH2263, which were monomorphic, without homozygotes, and unable to be amplified, respectively. In general, the closer the markers are to GALK1 and TK1, the more significant the allelic asso-

Figure 1—P values plotted as –log10(P) versus markers at their map locations on canine chromosome 9. Notice that –log10(P) values increase (ie, actual P values decrease) with increasing proximity to the GALK1 and TK1 loci, indicating the most likely position of the gene locus for progressive retinal atrophy.

Therefore, these data support the hypothesis that PRA in American Eskimo Dogs is the progressive rod-cone degeneration form seen in numerous other breeds, making it unnecessary to collect data from the remaining MSS-2 markers. These findings allow for the development of a microsatellite marker-based test to identify carrier (unaffected) and affected dogs before clinical signs become apparent. This is a noninvasive test that can be performed at an early age and provide breeders with the information needed to reduce the incidence of PRA in their lines. A caveat to the use of this marker-based test is that no flanking markers in proximity and also linked to PRA were identified. Clinically normal homozygous dogs for the alleles linked with PRA were observed; thus, caution should be exercised when interpreting the results of this test. Finally, these data facilitate identification of candidate genes for positional cloning and eventual development of a mutation-based test.

References