Mapping the Mutation Causing Lens Luxation in Several Terrier Breeds

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Abstract

Primary lens luxation (PLL), a painful and blinding inherited condition, is common in several breeds of terrier. Here we have examined the Veterinary Medical Database of patient encounters and Canine Eye Registration Foundation (CERF) cases records for the last 10 years and found the diagnosis recorded in 85 breeds. We have performed association analysis using a genome-wide microsatellite screen to map mutations underlying the condition in miniature bull terriers and Lancashire heelers. These studies show microsatellite alleles in disequilibrium with disease status with highest support in a 6.3-Mbp region in the central part of chromosome 3 ($-\log P_{\text{max}} = 6.4$). The same region also shows an association to the disease in Tibetan terriers. Tight junction protein-1 ($TJP1$) is a positional candidate to contain the PLL mutation. All recognized exons and splice junctions of $TJP1$ have been sequenced from affected, obligate carrier and control Lancashire heeler dogs. Several polymorphisms have been found, but these are not likely to cause the disease.

Methods

Dogs and DNA Samples

All dogs used in the study were dogs from the UK pet population that had been examined by qualified veterinary ophthalmologists and either had a documented history of idiopathic bilateral lens luxation or had eyes tested as normal within the previous 12 months. All control dogs used were over 7 years of age. Blood samples (2–5 ml) were collected and preserved in EDTA. DNA was extracted using the Nuclon genomic DNA extraction kit (Tepnel, Manchester, UK) according to the manufacturer’s instructions.

Genotyping

Three hundred and sixty-four microsatellite markers from all autosomes were used in a scan for association between the
marker and PLL (tabulated in supplementary information, Table S1). New markers in a supported region of chromosome 3 were microsatellites picked at approximately 1-Mb intervals (using the CanFam 2.0 build of the canine genome), with primers selected using Primer 3 software (Rozen and Skaletsky 2000). DNA samples of 5–10 ng were amplified and labeled using published conditions (Mellersh et al. 2006) or in the case of new markers (Table 1) by indirect labeling incorporating a fluorescently labeled M13 primer as well as forward and reverse primers for 1 min at 95°C, 1 min at 54°C, and 1 min at 72°C for 30 cycles. Labeled PCR fragments were analyzed on an ABI 3100 analyzer or for some indirectly labeled markers, on a Beckman CEQ8000. Results were analyzed with GENEMAPPER 3.5 software, and association was tested by comparing allele frequencies at each marker position of PLL-affected animals with those of obligate carriers, with control non–PLL-affected animals or with a joint group containing both obligate carriers and controls. CLUMP v23 software was used to simulate $\chi^2$, comparing each allele independently, to analyze the population split as a single allele and a clump containing all other alleles using each allele in turn as a singleton, or to analyze all possible allele combinations in a $2 \times 2$ table. Initially, $10^6$ simulations were used for each marker and trial. Where an association was detected, $10^7$ simulations were used to obtain a $P$ value. (The original CLUMP software is described in Curtis and shan 1995, see also Sham and Curtis 1995; Curtis 2005.)

Sequencing the Tight Junction Protein-1 Gene

Exons and splice junctions of the tight junction protein-1 (TJP1) gene were amplified using primers selected as before to lie in introns 50–100 bp from intron/exon boundaries or within the larger exons (Table 1). DNA from 2 PLL-affected, 2 obligate carrier, and 2 normal Lancashire heeler dogs was sequenced by standard methods. Normal dogs were unrelated to affecteds at 3 generations, were over 7 years old, and were ophthalmologically normal.

Results

Breed Predispositions to PLL

The Veterinary Medical Databases (compiled as SNVDO and SNOMED records) and the CERF database covering 1996–2005 were interrogated for cases diagnosed as PLL (Table 2). Two thousand two hundred and fifty-two cases of PLL were recorded in 85 canine breeds. The CERF database is an open registry for eye examination results from ACVO diplomates, whereas the other 2 databases are repositories for patient encounters from nearly all North American veterinary schools. For the 2 patient encounter databases, 1924 PLL cases amounted to 0.48% of all cases in the databases over the period. Many of the breeds with high relative risk have been previously reported, such as the Shar-Pei and

### Table 1. Relative risk of primary lens luxation by breed

<table>
<thead>
<tr>
<th>Breed</th>
<th>VetMed (SNVDO)</th>
<th>VetMed (SNOMED)</th>
<th>CERF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (total)</td>
<td>RR CI 95</td>
<td>RR CI 95</td>
</tr>
<tr>
<td>American cocker spaniel</td>
<td>286</td>
<td>3.74 3.25–4.30</td>
<td>5.61 4.06–7.74</td>
</tr>
<tr>
<td>Australian cattle dog</td>
<td>29</td>
<td>3.34 2.32–4.80</td>
<td>1.61 0.60–4.29</td>
</tr>
<tr>
<td>Bearded collie</td>
<td>11</td>
<td>5.25 2.37–11.58</td>
<td>0.00 —</td>
</tr>
<tr>
<td>Boston terrier</td>
<td>64</td>
<td>4.46 3.44–5.77</td>
<td>1.05 0.34–3.26</td>
</tr>
<tr>
<td>Britany spaniel</td>
<td>32</td>
<td>2.13 1.36–3.33</td>
<td>5.18 2.87–9.35</td>
</tr>
<tr>
<td>Bull terrier</td>
<td>17</td>
<td>1.36 0.51–3.62</td>
<td>11.50 6.16–20.71</td>
</tr>
<tr>
<td>Fox terrier</td>
<td>42</td>
<td>6.26 4.41–8.89</td>
<td>12.71 7.15–22.61</td>
</tr>
<tr>
<td>Italian greyhound</td>
<td>23</td>
<td>1.78 0.67–4.73</td>
<td>1.30 0.18–9.24</td>
</tr>
<tr>
<td>Jack Russell terrier</td>
<td>146</td>
<td>5.87 4.66–7.39</td>
<td>4.41 2.77–7.05</td>
</tr>
<tr>
<td>Miniature bull terrier</td>
<td>15</td>
<td>N/A —</td>
<td>N/A —</td>
</tr>
<tr>
<td>Mixed-breed dog</td>
<td>388</td>
<td>0.95 0.84–1.07</td>
<td>0.99 0.77–1.28</td>
</tr>
<tr>
<td>Old English sheepdog</td>
<td>15</td>
<td>3.66 2.17–6.17</td>
<td>0.00 —</td>
</tr>
<tr>
<td>Poodle, miniature</td>
<td>111</td>
<td>4.51 3.67–5.55</td>
<td>5.46 3.29–9.08</td>
</tr>
<tr>
<td>Poodle, toy</td>
<td>71</td>
<td>3.10 2.37–4.06</td>
<td>5.45 3.28–9.06</td>
</tr>
<tr>
<td>Rat terrier</td>
<td>24</td>
<td>4.83 2.69–8.68</td>
<td>12.45 7.17–21.64</td>
</tr>
<tr>
<td>Shar-Pei</td>
<td>86</td>
<td>7.18 5.66–9.12</td>
<td>10.79 6.44–18.07</td>
</tr>
<tr>
<td>Tibetan terrier</td>
<td>12</td>
<td>4.94 1.87–13.04</td>
<td>5.45 0.79–37.44</td>
</tr>
<tr>
<td>Toy fox terrier</td>
<td>10</td>
<td>8.26 4.48–15.19</td>
<td>0.00 —</td>
</tr>
<tr>
<td>Welsh terrier</td>
<td>8</td>
<td>5.74 2.76–11.93</td>
<td>0.00 —</td>
</tr>
<tr>
<td>Whippet</td>
<td>20</td>
<td>3.28 1.65–6.54</td>
<td>0.00 —</td>
</tr>
<tr>
<td>Total numbers</td>
<td>2252</td>
<td>1500 334</td>
<td>328</td>
</tr>
</tbody>
</table>
the Chinese crested dog, but several were not, including popular breeds such as the American cocker spaniel and miniature and toy poodles. PLL has highest recorded incidence in a number of terrier breeds including the bull terrier/miniature bull terrier (definition of these 2 breeds varies between VMDB and CERF database), fox terrier and toy fox terrier, Jack Russell terrier, rat terrier, and Tibetan terrier.

Mapping the PLL Locus in Miniature Bull Terriers and Lancashire Heelers

In an initial screen, microsatellite markers located across all autosomes were amplified from genomic DNA from 23 PLL-affected miniature bull terriers, together with an equal number of obligate PLL carriers with no luxation, and also 23 PLL-affected Lancashire heeler, with an equal number of obligate carriers with no luxation. Preliminary analyses for each breed showed a region of decreased heterozygosity in chromosome CFA3, with a proportion of markers between 24.5 Mb and 46.4 Mb showing \(-\log{P}\) values above 3.0. New markers were added at 1-Mb intervals from 38 to 45 Mb, and analysis was performed on groups of 45 miniature bull terriers with PLL together with 24 miniature bull terriers obligate carriers (no PLL) and 24 other control miniature bull terriers (no PLL), as well as on 44 PLL-affected Lancashire heelers with 41 obligate carriers (no PLL) and 27 other control Lancashire heelers (no PLL). For each breed, an association was found (Figure 1), with a maximum \(-\log{P}\) of 5.7 (miniature bull terrier) or 6.4 (Lancashire heeler), in both cases associated with the marker ZuBeCa4 at 43.0 Mb. No variable microsatellite marker used showed complete homozygosity in all affected dogs. Shared microsatellite haplotypes and homozygosity in most affected dogs extended from CamC03.043 (40.1 Mbp) to FH2145 (46.4 Mbp) in Lancashire heeler and from CamC03.044 (40.9 Mbp) to FH2145 in miniature bull terrier (Table 2 gives commonest allele frequencies). CamC03.043 has rather low heterozygosity in the Lancashire heeler, so that the \(-\log{P}\) value is not high. The analyzed dogs are not sufficiently related to allow the identification of critical recombinations which would further refine this interval.

The same set of markers were studied for association in 12 PLL-affected and 12 obligate carrier Tibetan terriers. Affected animals were more often homozygous than carriers across all markers tested (from 35.2M–46.4 M). At CamC03.046, all affected animals were homozygous for a single allele, whereas the carrier group included 2 homozygotes and 9 heterozygotes for this allele as well as a single animal that did not have the allele on either chromosome (\(-\log{P}\approx3.87\)). A preliminary study of 2 affected and 4 normal Jack Russell terriers and of 1 affected and 2 normal Parson Russell terriers suggested homozygosity among the PLL affecteds but not the normals between 41.9M and 46.4M.

Sequencing Study of TJP1

Exons and exon intron junctions of \(TJP1\) were amplified by PCR and sequenced from 2 PLL-affected, 2 obligate PLL carrier, and 2 unrelated normal Lancashire heeler. The normal Lancashire heelers were clinically clear of PLL and were homozygous for non–disease-associated alleles of CFA3 microsatellites. Six polymorphisms were found including one nonsynonymous substitution in coding sequence (C642T; A > V) (Table 3). For the polymorphisms observed here, one obligate carrier was homozygous for a haplotype shared with both affecteds, whereas the other carrier and both control dogs were heterozygous for other haplotypes.

Discussion

This study has defined an interval of 5.5 Mb on chromosome 3 containing a mutation associated with PLL in the miniature bull terrier and the Lancashire heeler dog. Initial evidence also suggests that a mutation in the same interval causes the disease in other terrier breeds including the Parson and Jack Russell terriers and the less closely related Tibetan terrier. The interval contains 33 genes represented at mRNA, expressed sequence tag, or protein level and a further 37 modeled by the Gnomon gene prediction program (National Center for Biotechnology Information databases). We are now reducing the mutation-containing interval by increasing the number of dogs used in our studies and also the number of breeds in use.

The facts that the same interval appears to be linked to PLL in several different terrier breeds and that a microsatellite haplotype is shared by PLL-affected Lancashire heelers and miniature bull terriers indicate that the causal mutation is probably very old, having arisen in a common ancestor of all these related breeds. Although it is likely that some of the recorded cases on the VetMed databases were in fact secondary to traumatic injury or some other cause, those breeds
in which incidence is high are likely to have an inherited predisposition to PLL. The large number of such breeds represents further circumstantial evidence for an ancient mutation, although alternative explanations are clearly possible (mutations at multiple loci can cause the disease or a single highly mutable gene is involved). PLL is considered to be recessively inherited in the Tibetan terrier (Willis et al. 1979), and inheritance is consistent with a recessive condition in other breeds as well. However, the prevalence of PLL in mixed-breed dogs, and especially in terrier crosses, indicates that the condition might have a dominant but partially penetrant mode of inheritance against some genetic backgrounds (in some breeds or individuals). At the moment, we cannot rule this out even in the Lancashire heeler or miniature bull terrier using the genotyping data presented here, as no highly polymorphic marker has yet been found that is homozygous in all affected dogs. Alternatively, different mutations in the same gene could cause recessive and fully dominant forms of PLL. This scenario is not at all unusual for many monogenic disorders. For instance, recently different mutations in a single gene were reported to cause recessive and dominant forms of hereditary cataract in different breeds of dog (Mellersh et al. 2006).

The \textit{TJP1} (previously \textit{ZO-1}) gene is within the disease interval for both miniature bull terrier and Lancashire heeler and is a potential candidate for this disease based on the localization of its protein product at the equatorial attachment areas of lens to the zonule and at the lateral cell borders of

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
Exon Position & Alleles & \multicolumn{3}{c|}{Affected} & \multicolumn{3}{c|}{Carrier} & Clear \\
\hline
1 & 19 bp before exon start & C & T & C & C & C & C/T & C/T & C/T \\
6 & 57 bp before exon start & TTA & TTA deletion & TTA & TTA & TTA deletion & TTA & TTA deletion & TTA deletion \\
10 & 50 bp after exon end & G & A & G & G & G & G & G/A & G/A \\
15 & 43 bp before exon start & G & A & A & G & G/A & G & A & A \\
20 & 60 bp before exon start & & & & T insertion & T insertion & & & \\
20 & 642 bp in exon & C & T (A > V) & C & C & C & C/T & C/T & C/T \\
\hline
\end{tabular}
\caption{Polymorphisms in \textit{TJP1}}
\end{table}
RPE and ciliary process cells (Singh et al. 2001; Tserentsoodol et al. 1998; Nguyen et al. 2005) and also on its central role as a “scaffold protein” in the formation of epithelial tight junctions in binding JAM1 and F-actin (Itoh et al. 1997; Liu et al. 2000). Several polymorphisms were found in the TJP1 gene in Lancashire heeler, including one nonsynonymous mutation in coding sequence. However, the change seen is conservative, and the C allele found in the boxer sequence (CanFam 2.0) is homozygous in PLL dogs, whereas the T allele is seen heterozygously in one of two obligate carrier dogs and in dogs from the general population. Boxers have not been recorded to suffer PLL, so it is rather unlikely that this is a causative mutation for the disease. None of the other polymorphisms we have found in TJP1 are unambiguously likely to alter production or function of the gene product, making it less likely that mutations in TJP1 cause PLL. However, studies are ongoing to investigate the intronic and promoter regions of TJP1, while TJP1 is known to undergo differential splicing, so that there is also a possibility that ocular specific exons exist and have not yet been identified or analyzed.

Supplementary Material
Supplementary Table S1 can be found at http://www.jhered.oxfordjournals.org/.

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Veterinary Medical Databases [Internet]. Association of Veterinary Medical Data Program Participants Inc. [cited 2006 March 1]. Available from: http://www.vmdbl.org/.

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