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NO EVIDENCE OF LINKAGE BETWEEN 35 GENETIC MARKERS
AND THE SPIDER LAMB SYNDROME GENE IN SHEEP

by

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No Evidence of Linkage Between 35 Genetic Markers and the Spider Lamb Syndrome Gene in Sheep

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Abstract

Spider Lamb Syndrome (SLS) is a semi-lethal congenital disorder affecting the Suffolk and Hampshire sheep breeds. The clinical manifestations of the syndrome include severe skeletal and muscular abnormalities with long, fine-boned legs, curvature of the spine and nasal septum, minimal muscling and severe muscle atrophy. The affected animals have a difficult time walking and most lambs do not survive past the first six months of life. A single autosomal recessive gene causes the disorder. Researchers believe the SLS gene arose as a single source mutation in a popular Suffolk blood line in the 1970s. Heterozygous carriers of the SLS gene appear normal in phenotype and currently can only be identified through progeny testing. If researchers could identify a genetic marker that cosegregates with the SLS locus, this marker could be used for low-cost carrier screening. In this study, we tested 35 bovine and ovine dinucleotide microsatellite markers as possible markers for the Spider Lamb Syndrome gene. No strong evidence for linkage between the tested microsatellite markers and the SLS gene was found.

KEY WORDS: Bovine, Ovine, Spider Lamb Syndrome, Genetic Marker.

Introduction

Ovine hereditary chondrodysplasia, more commonly known as Spider Lamb Syndrome (SLS), is a semi-lethal congenital disorder caused by a single autosomal recessive gene (Berg et al., 1987; Thomas and Cobb, 1986; Vanek et al., 1986). Researchers believe the disorder arose
as a single source mutation in a popular Suffolk blood line in the 1970s (Russell et al., 1989). The mutation has since spread through both the Suffolk and Hampshire breeds (Vanek, 1986).

Typical clinical signs of the disorder include humped and twisted spines; crooked, fine-boned, and disproportionately long limbs; an angled pelvis with shallow torso dimensions; and the nasal septum appears markedly curved. The affected animals show minimal muscling and with age the skeletal abnormalities and extreme muscle atrophy become more apparent. At necropsy the most commonly observed lesions include a medial deviation of the forelimb, a medial deviation of the tarsus, and pathological fractures of the hock bone or upper thigh bone. The affected vertebrae, elbow joints, and physes of long bones each have excess cartilage at the growth plates. In the majority of cases, the affected joints appear arthritic with multiple bony growths.

Radiographic evaluations show separate islands of ossification in subchondral regions and abnormal endochondral ossification is consistently reported in the elbows, shoulders and sternum. Radiographs of the growth plates show either prematurely closed or open and deteriorating growth plates (Vanek et al., 1986; Rook et al., 1986). In the distal radial physis of spider lambs, there is a less ordered appearance to the columns of chondrocytes (Figures 1 & 2) (Troyer et al., 1988). Because the disorder is a syndrome, not all of the symptoms may be present and the symptoms may be present at birth or take as long as 3 months to appear.

Some of these symptoms are very similar to an inherited cartilage defect in the Suffolk breed known as arthrogryposishydranencephaly (AGH). However, the AGH syndrome has a primary problem with the central nervous system, whereas Spider Lamb Syndrome affects primarily just the muscular and skeletal systems (Rook, 1987).

Clinicopathologic evaluations have shown that there is little difference in blood count,
serum chemistry profile and serum nutritional assays between spider lambs and normal lambs. However, a trend towards elevated skeletal and muscle enzymes in the blood was noted in spider lambs. Bovine virus diarrhea and bluetongue virus-neutralization titers were determined as well as a complement fixation for *Chlamydia* but no serological differences were found between normal and spider lambs (Rook et al., 1987).

Collagen types I, II, and III are known as the fibrillar collagens because they form supra molecular aggregates (fibrils) in several connective tissues. Abnormalities in the structure and function of fibrillar collagens have been associated with Marfan syndrome, Ehlers-Danlos syndromes, osteogenesis imperfecta, and the chondrodystrophies (Tsipouras and Ramirez, 1987). Biochemical analysis of the tissues from both normal and spider affected lambs indicated lower concentration levels of collagen and proteoglycan in spider affected lambs than in normal lambs (Nakano et al., 1994). SDS-PAGE analysis of collagen extracted from spider affected bone revealed type I collagen normal in appearance with no type III collagen present (Troyer et al., 1988). Troyer et al. (1988) hypothesizes that the biochemical abnormalities in spider affected lambs could result from a defective extracellular matrix molecule, the presence of an extracellular matrix molecule that is not usually present, or a collagen abnormality within the growth plate cartilage.

Insulin-like growth factors and their binding proteins function as important regulators of bone growth. Osborne et al. (1991) investigated the possible endocrine involvement of IGF-I and IGF-BP to determine if circulating levels were different in spider lambs as compared to normal lambs and to determine if the expression levels of IGF-I and -II were different in the liver and muscle cells of spider lambs. The study showed that the spider affected lambs had a 37%
lower level of circulating IGF-I and a 61% higher level of the 32 kDa binding protein than normal
controls. The gene expression of IGF-I and -II was not different in the muscles but IGF-I
expression in the liver of spider affected lambs was nearly double that of control animals.

**Significance**

Producers have turned away from the once popular Suffolk breed because of the severe
economic loss caused by the presence of the SLS gene in a flock. Sheep carrying one normal
allele and one spider allele have a normal phenotype but when mated to another carrier there is a
25% probability that the offspring will be affected with Spider Lamb Syndrome. Currently the
only way to identify carriers is through progeny testing which is expensive, time-consuming, and
not always reliable. If a genetic marker that cosegregates with the spider allele was identified, this
marker could be used for low-cost carrier screening. The marker could be the SLS gene itself or
a marker that lies very close to the gene on the chromosome, although there could be reshuffling
of the marker and the SLS gene. A test for the presence of the marker could be determined on a
single blood sample taken from the animal in question.

Researchers here at USU have begun the search for such a marker. Shay *et al.* (1993)
selected collagenase, insulin-like growth factor I, and fibrillin as candidate genes for the
syndrome. The researchers detected polymorphisms in these genes through restriction fragment
length polymorphism (RFLP) analysis. Random genetic markers, including 21 dinucleotide
microsatellites and 4 locus-specific variable number of tandem repeats (VNTRs), were also tested
in their study. Linkage between these markers and the SLS gene was tested but unfortunately no
evidence of linkage was found. Jenson *et al.* (1994) continued with this work and tested linkage
between the SLS gene and 2 locus-specific VNTRs and 4 dinucleotide microsatellites. Again,
there was no evidence of linkage between these 6 markers and the SLS gene.

**Materials and Methods**

The research objective of this study was to identify a genetic marker for the SLS gene using microsatellite analysis. Microsatellite primers flank areas of dinucleotide tandem repeats which exist throughout the genome of the animal. The number and length of these repeats becomes widely variable in a population and therefore a population can be very polymorphic for these markers. The tandem repeat area is amplified using the polymerase chain reaction (PCR). During PCR, $P^{32}$ is incorporated into the newly synthesized DNA strands. The samples are run on a polyacrylamide electrophoretic gel (PAGE) and exposed to X-ray film so that the different “alleles” existing in a population can be visualized.

The marker is then tested for association with the SLS gene using linkage analysis. In this analysis, the null hypothesis is that the marker exists on a different chromosome from the chromosome containing the SLS gene or the SLS gene and the marker may be on the same chromosome but they are too far apart for linkage to be detected. If the SLS gene and the selected marker are not linked, the SLS gene and the marker independently segregate in a double heterozygous animal and produce all four possible combinations of gametes in equal proportions. However if the marker is tightly linked to the SLS gene, then only two possible gametes can be produced, unless crossing over has resulted between the marker and the SLS gene in which case recombinant gametes will be formed. Because of the possibility of recombination, all four gamete classes can occur but linked genes will yield greater than 50% parental type gametes and less then 50% recombinant type gametes. The exact frequency of each type varies according to the distance between the marker and the gene. If they are close together, very few recombinant type
gametes will form through crossing over, whereas, if they are further apart, a larger proportion of recombinants will form.

USU currently maintains a research sheep flock with known carriers of the SLS gene. Using this flock a pedigree consisting of one heterozygous carrier ram, 40 heterozygous carrier dams, and 65 offspring has been established. Seventeen (26%) of these offspring are affected with the Spider Lamb Syndrome.

Approximately 30 mL of whole blood was collected from each animal in the flock and, using a saturated salt/ethanol precipitation procedure adapted from Miller et al. (1988), pure genomic DNA was extracted. The DNA from each animal was diluted to 0.02 mg/mL with distilled water and then genotyped for the microsatellite as described above.

Random genetic microsatellite markers were chosen by first screening bovine and ovine microsatellite primers purchased from Research Genetics (Huntsville, AL). These primer sets flank tandem dinucleotide repeat areas (markers) in the genome and have been mapped to a specific chromosomal region in either the bovine or ovine genome. After determining the markers that amplified ovine DNA, the carrier ram was genotyped with these markers. All the markers for which the ram was heterozygous were then tested on the 17 spider affected lambs in a two-point test cross. Because the 17 offspring are spider affected, we know that (at least for these offspring) the ram contributed his SLS allele rather than his normal allele. Looking at the genotypes of the spider affected animals for these markers, we identified how many times the ram (which is heterozygous for the marker) contributed one of his marker alleles and how many times he contributed his other marker allele. Offspring with the same marker genotype as the ram were noninformative because we could not determine which allele the ram contributed. If the ram
passed each allele with equal frequency, then there was no evidence of linkage between this
marker and the SLS gene, but if one allele was at a significantly higher frequency in the offspring
than the other allele (for example, 12 of the offspring received one allele while 5 of the offspring
received the other allele) then this marker warranted further investigation.

Markers showing unequal allele segregation among the affected offspring were then tested
on the entire pedigree. Linkage analysis between the Spider Lamb Syndrome locus and the
genetic markers was preformed using the computer linkage program, LINKAGE, which
generated Lod scores at different recombination rates between the marker and the SLS locus. If
the calculated Lod score was greater than 3, then the null hypothesis (the marker is not linked to
the SLS locus) would be rejected.

**Results and Discussion**

Lod scores for linkage of microsatellite markers to the Spider Lamb Syndrome locus are given in
the following table:

<table>
<thead>
<tr>
<th>Marker</th>
<th>Ovine Chromosome</th>
<th>Maximum Lod Score</th>
<th>Recombination Fraction ($\phi$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM6438</td>
<td>1</td>
<td>-0.11</td>
<td>0.40</td>
</tr>
<tr>
<td>OarDB6</td>
<td>1</td>
<td>-0.11</td>
<td>0.40</td>
</tr>
<tr>
<td>MAF64</td>
<td>1</td>
<td>-0.18</td>
<td>0.40</td>
</tr>
<tr>
<td>CSSM19</td>
<td>1</td>
<td>-0.11</td>
<td>0.40</td>
</tr>
<tr>
<td>INRA11</td>
<td>1</td>
<td>-0.06</td>
<td>0.40</td>
</tr>
<tr>
<td>TGLA10</td>
<td>2</td>
<td>0.00</td>
<td>0.40</td>
</tr>
<tr>
<td>TGLA377</td>
<td>2</td>
<td>0.25</td>
<td>0.30</td>
</tr>
<tr>
<td>ETH10</td>
<td>3</td>
<td>-0.14</td>
<td>0.40</td>
</tr>
<tr>
<td>CSSM34</td>
<td>3</td>
<td>-0.01</td>
<td>0.40</td>
</tr>
<tr>
<td>RM188</td>
<td>4</td>
<td>-0.21</td>
<td>0.40</td>
</tr>
<tr>
<td>McM218</td>
<td>4</td>
<td>0.07</td>
<td>0.40</td>
</tr>
<tr>
<td>RM67</td>
<td>4</td>
<td>0.06</td>
<td>0.40</td>
</tr>
<tr>
<td>OarCP26</td>
<td>4</td>
<td>0.07</td>
<td>0.40</td>
</tr>
<tr>
<td>TGLA137</td>
<td>5</td>
<td>0.79</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Lod scores for linkage of microsatellite markers to the Spider Lamb Syndrome locus (cont.):

<table>
<thead>
<tr>
<th>Marker</th>
<th>Ovine Chromosome</th>
<th>Maximum Lod Score</th>
<th>Recombination Fraction ((\phi))</th>
</tr>
</thead>
<tbody>
<tr>
<td>McM527</td>
<td>5</td>
<td>0.41</td>
<td>0.30</td>
</tr>
<tr>
<td>BM1853</td>
<td>5</td>
<td>0.65</td>
<td>0.20</td>
</tr>
<tr>
<td>OarJMP8</td>
<td>6</td>
<td>0.91</td>
<td>0.30</td>
</tr>
<tr>
<td>OarAE64</td>
<td>7</td>
<td>-0.09</td>
<td>0.40</td>
</tr>
<tr>
<td>RM11</td>
<td>8</td>
<td>-0.10</td>
<td>0.40</td>
</tr>
<tr>
<td>BM2504</td>
<td>8</td>
<td>-0.18</td>
<td>0.40</td>
</tr>
<tr>
<td>BM1227</td>
<td>8</td>
<td>-0.04</td>
<td>0.40</td>
</tr>
<tr>
<td>MAF33</td>
<td>9</td>
<td>-0.02</td>
<td>0.40</td>
</tr>
<tr>
<td>OarAE16</td>
<td>13</td>
<td>-0.15</td>
<td>0.40</td>
</tr>
<tr>
<td>BM848</td>
<td>15</td>
<td>0.07</td>
<td>0.40</td>
</tr>
<tr>
<td>FSHB</td>
<td>15</td>
<td>-0.14</td>
<td>0.40</td>
</tr>
<tr>
<td>BR3510</td>
<td>15</td>
<td>-0.16</td>
<td>0.40</td>
</tr>
<tr>
<td>BM4107</td>
<td>16</td>
<td>0.28</td>
<td>0.30</td>
</tr>
<tr>
<td>BM1225</td>
<td>16</td>
<td>-0.11</td>
<td>0.40</td>
</tr>
<tr>
<td>MAF209</td>
<td>17</td>
<td>0.19</td>
<td>0.30</td>
</tr>
<tr>
<td>OarHH56</td>
<td>20</td>
<td>-0.19</td>
<td>0.40</td>
</tr>
<tr>
<td>JP15</td>
<td>21</td>
<td>1.11</td>
<td>0.20</td>
</tr>
<tr>
<td>OarCP20</td>
<td>21</td>
<td>-0.17</td>
<td>0.40</td>
</tr>
<tr>
<td>OarVH72</td>
<td>25</td>
<td>-0.06</td>
<td>0.40</td>
</tr>
<tr>
<td>BM203</td>
<td>26</td>
<td>-0.19</td>
<td>0.40</td>
</tr>
<tr>
<td>BMC1260</td>
<td>unassigned</td>
<td>0.04</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Because none of the generated lod scores is greater than three, there is no evidence that these microsatellite markers are linked to the Spider Lamb Syndrome locus. It is clear that in the future additional random markers as well as candidate genes should be analyzed for linkage to the SLS locus.

With persistence, a marker at a significant recombination rate will eventually be found and then, using ovine genome maps, markers at even smaller recombination rates (closer to the SLS locus) can be identified. Through this method, a genetic marker for carrier screening will be
developed. However, the research does not have to end here. Once the location is known, the SLS gene can be cloned and sequenced and the information gained from these projects will aid in understanding what causes the debilitating physical state of the lambs it affects.

Acknowledgments:

The authors wish to thank Wendy Thomas, Josie Miller, Catherine Larsen, and Dave Forrester for their assistance in this project.
Literature Cited in Text


Figure 1. Distal radial physis from a normal lamb.

Figure 2. Distal radial physis from a spider affected lamb.

Notice the appearance of the chondrocytes in each figure.

Figures from Troyer et al. (1988).