ABSTRACT: Entropion is an inversion of the eyelid allowing direct contact between the eyelashes and cornea, potentially causing blindness if not treated. In domestic sheep, entropion has a variable frequency (0-80%) worldwide and is heritable (heritability 0.08-0.21). Identification of genes associated with entropion could facilitate development of genetic markers to select against entropion. Therefore, a genome-wide association scan was conducted with 998 Columbia, Polypay, and Rambouillet sheep genotyped using the Illumina OvineSNP50 BeadChip. Entropion status was recorded within 48 hours of birth and overall prevalence was 5.6%. Data was analyzed using logistic regression models in PLINK v1.06 that accounted for breed, PPC clusters, and SNP minor allele. One genome-wide significant (P<1x10^-6) SNP was identified on chromosome 6 and five SNP were genome-wide suggestive (P<1x10^-8) on chromosomes 1, 2, 13 and 16. We are evaluating these regions to identify the underlying causal mutations.

Keywords: Domestic sheep; Entropion; Genome-wide association study

Introduction

The ability to use genomic markers to select for production or disease traits in sheep is a growing field of research. Entropion is a condition in which the eyelid rolls toward the eye, causing eyelashes to contact the cornea (Warwick and Berry, 1962) and occurs in wide variety of mammalian species (humans, Zagora, 1966; dogs, Menges, 1946; horses, Peiffer et al., 1977; sheep Warwick and Berry, 1962). In lambs, entropion can affect 1 or both eyes, both sexes, and generally the lower eyelid (Ellis, 1943; Joyce, 1981). Entropion was found to be heritable (0.08-0.21) in 5 breeds of sheep (Sakul and Kellom, 1996) and has a variable within flock worldwide frequency (1.1-80%; Green et al., 1995). While there are varying methods to correct entropion (Sakul et al., 1996), intervention can be labor-intensive and financially prohibitive. However, development of a genetic test would provide producers the opportunity to use marker assisted selection to eliminate entropion within their flocks.

The Ovine SNP50 BeadChip was developed by the International Sheep Genome Consortium (Kijas et al., 2012) as a tool to identify genomic regions associated with phenotypic traits of interest. This technology has been used successfully to identify genomic regions associated with coat color (Kijas et al., 2013), inherited disease conditions (Becker et al., 2010) erythrocyte traits (Gonzalez et al., 2013), parasite infection (Silva et al., 2012), and other infectious disease traits (Heaton et al., 2012; White et al., 2012). Therefore, this study examined genome-wide association with entropion in 3 common US sheep breeds.

Materials and Methods

Data. All animal care and handling procedures were reviewed and approved by the Washington State University Institutional Animal Care and Use Committee and/or by the U.S. Sheep Experiment Station (USSES) Animal Care and Use Committee. Entropion was defined as the inward rolling of 1 or both lower eyelids within 48 hours of birth by trained technicians. Entropion was recorded as present or absent for all sheep born alive at the U.S. Sheep Experiment Station. Sheep sampled for this study were ewes aged 1-5 years of Rambouillet (n=414), Polypay (n=438) and Columbia (n=146) breeds. These ewes were produced from 58, 49, and 40 unique sires and 350, 338, and 115 different dams per Rambouillet, Polypay and Columbia breed, respectively. Entropion was documented in 59 of these ewes, with all breeds represented.

Genotyping. Blood was collected via jugular venipuncture into EDTA-coated vacutainer tubes. Blood was processed for peripheral blood leukocytes and DNA isolated using the Invitrogen GeneCatcher gDNA 3-10ml Blood kit per manufacturers’ instructions (Life Technologies, Carlsbad, CA; Herrmann-Hoesing et al., 2007). Genotyping services were provided by Geneseek Inc. (Lincoln, NE) using the OvineSNP50 Infinium BeadChip (Illumina Inc., San Diego, CA).

Statistical analyses. Samples with high genotype call rates (>97%) were retained for analysis. Multidimensional scaling and pairwise population concordance clustering were performed as previously described (White et al., 2012), and resulting breed groups were used for categorical stratification in association analysis. The presence or absence of entropion eyelid was analyzed using logistic regression models in PLINK v1.06 (Purcell et al., 2007) that accounted for breed and PPC clusters as well as the SNP minor allele. Screening criteria for SNP inclusion in PLINK analysis were set as previously described (White et al., 2012): missingness by individual (0.1), missingness by marker (0.03), minor allele frequency (0.01), and Hardy-Weinberg equilibrium (0.000001, which corresponds to P=0.05 after Bonferroni correction for 50,000 marker tests). Inheritance models used included additive allelic, recessive,
dominant, and genotypic. Permutations were calculated within sire family as previously described (White et al., 2012), and 1000 permutations were used to establish significance. Genome-wide significance was defined by empirical P<0.05. Genome-wide suggestive status was defined by nominal P<1x10^{-5} (Hindorff et al., 2009).

Results and Discussion

From the original set of 998 ewes, sample quality control criteria and outlier status, when evaluating breed, reduced the total number of sheep evaluated to 964. The remaining ewes included Rambouillet (n=399), Polypay (n=423), and Columbia (n=142). The average genotyping call rate was 98.06% for the remaining sheep. Genome-wide association with entropion identified six unique SNP, one which was genome-wide significant on chromosome 6 and five that were genome-wide suggestive on chromosomes 1, 2, 13, and 16 (Table 1). Q-Q plots with observed vs expected P-value distributions were generated with and without accounting for the top six SNP (Figure 1). Plot A of Figure 1 shows some apparent population stratification in the divergence of observed P-values from the expected line. This could be due to differing frequencies of a small number of genetic mutations, and Figure 1 B shows the elimination of almost all apparent population stratification after accounting for the top six SNP. Plot B also demonstrates that our statistical model fit our data appropriately. For each SNP the best-fitting mode-of-inheritance association model, odds ratio, genes located near the identified SNP are reported (Table 1). The odds ratio for the SNP on chromosome 6 is an impressive 37.47, where an odds ratio above 2 is considered large in human diagnostic testing (Vassy et al., 2012). This SNP is located within solute carrier 2 C 9 (SLC2A9, a.k.a. GLUT9). This gene is a glucose, fructose, and urate transporter expressed in multiple tissues including chondrocytes (Mobasher et al., 2002; Mueckler and Thorens, 2013). Contraction of the subepithelial fibrous membrane formed by vertically oriented parallel collagen fibers has been found to contribute to entropion in humans (Al-Rajhi et al., 1993). The two SNP on chromosome 1 that were genome-wide suggestive were adjacent to one another and were counted as one genomic locus. Phosphoinositide 3-kinase beta (PIK3CB) is found on the outer membrane of eukaryotic cells and may modulate cell morphology (Blajecka et al., 2010). The chromosome 2 genome-suggestive SNP is located within Myosin IIB (MYO3B) which may play a role in adipose deposition (Fox et al., 2012). The chromosome 13 genome suggestive SNP is found within a voltage-gated potassium channel (KCNB1) which may impact cell volume (Walsh et al., 2001). The chromosome 16 genome suggestive SNP, with an odds ratio of 11.01, is relatively near neurolysin (NLN) a member of the metalloepidase M3 protein family which may be involved in the termination of the neurotensinergic signal in the central nervous system (Norman et al., 2003).

Table 1: Genomic regions associated with entropion.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Ch</th>
<th>Best fitting model</th>
<th>Nominal P-value</th>
<th>Odds Ratio</th>
<th>Genes within 40 Kb</th>
</tr>
</thead>
<tbody>
<tr>
<td>s65132</td>
<td>6</td>
<td>Rec</td>
<td>4.56x10^{-8}</td>
<td>37.47</td>
<td>SLC2A9+</td>
</tr>
<tr>
<td>OAR16_14874751</td>
<td>16</td>
<td>Dom</td>
<td>3.34x10^{-7}</td>
<td>11.01</td>
<td>NLN*</td>
</tr>
<tr>
<td>OAR1_268186998</td>
<td>1</td>
<td>Add</td>
<td>2.22x10^{-6}</td>
<td>3.18</td>
<td>PIK3CB+,</td>
</tr>
<tr>
<td>OAR1_268175642</td>
<td>1</td>
<td>Add</td>
<td>2.22x10^{-6}</td>
<td>3.18</td>
<td>PIK3CB*</td>
</tr>
<tr>
<td>s63760</td>
<td>13</td>
<td>Dom</td>
<td>2.93x10^{-6}</td>
<td>4.06</td>
<td>KCNB1+</td>
</tr>
<tr>
<td>OAR2_146760496</td>
<td>2</td>
<td>Rec</td>
<td>5.74x10^{-6}</td>
<td>4.27</td>
<td>MYO3B+</td>
</tr>
</tbody>
</table>

*Rec=Recessive, Dom= Dominance, Add=Additive
SNP located within gene
SNP located within 35kb of gene
Conclusion

Entropion is a condition in sheep that can lead to blindness if not treated. Identifying a marker or markers for marker assisted selection in sheep would assist producers in reducing the incidence of entropion within their flocks. This research identified five chromosomal regions that were associated with entropion in three breeds of sheep. Research is ongoing to identify the causal mutations within these genomic locations.

Literature Cited

Warwick, B. L. and Berry, R. O. (1962) J. Hered. 53:10-11.