Identification of Single Nucleotide Polymorphisms for Small Ruminant Health and Adaptation

Md. Rasel Uzzaman¹, Zewdu Edea², Rabiul Islam³, Kwan-Suk Kim⁴ and Mulumebet Worku¹*

¹Department of Animal Sciences, North Carolina Agricultural and Technical State University, 1601 East Market Street, Greensboro, NC 27411-0002, USA; muzzaman@ncat.edu
²Ethiopian Biotechnology Institute, Addis Ababa, P. O. Box. 5954, Ethiopia.
³Institute of Animal Science (IAS), Chinese Academy of Agricultural Sciences (CAAS), No. 2 Yuanmingyuan West Rd., Haidian, Beijing 100193, China.
⁴Department of Animal Science, Chungbuk National University, Cheongju, Chungbuk Province 28644, Republic of Korea.

*Correspondence: Professor, Department of Animal Sciences, North Carolina Agricultural and Technical State University, 1601 East Market Street, Greensboro, NC 27411-0002, USA Tel: 336-285-4816, E-mail: worku@ncat.edu

Abstract Production of small ruminant animals has increased globally in part because of increasing demand for meat and milk. According to the UN’s Food and agricultural organization (FAO), the estimated world’s population will approach 10 billion by the year 2050. Furthermore, the economic status of people in developing nations is improving. Therefore, increased production is required to meet nutritional requirements for food and fiber. There is a need to reduce the environmental impact and use of ineffective antibiotics and anthelmintics. Genomic tests, precision agriculture technologies in breeding, feeding or health drive sustainable productivity and treatment solutions. Genetic variation contributes to natural resistance and resilience of sheep and goats against parasites. Definition of genetic variation and understanding signatures of natural selection and association with disease is the best long-term solution and a benchmark to help improve breeds. Progress in genomics is allowing us to determine genetic variation at the whole animal level. Using Single Nucleotide Polymorphisms (SNP) analysis is a less costly approach to determine within breed or species variation between animals and adaption to an environment. It is important to identify immune gene variants that modulate host-pathogen interactions and are responsible for small ruminant health and immunity. We investigated SNP variants in selected genes related to innate immunity (Toll like receptors TLR and Galectins, LGALS) on 88 Ethiopian sheep genotyped with Ovine Infinium HD SNP BeadChip (600K) and 308 Chinese goats genotyped with Illumina Goat 50K BeadChip. An in-Silico approach with Ensembl database and SNP Chips was used to determine minor allele frequency. We identified 86 TLR and 19 LGALS SNP from sheep and 1 LGALS SNP in goats with variable minor allele frequency (MAF) level. This new knowledge and the SNP analysis approach can increase prediction of sheep and goat innate immunity and adaptation to control diseases for sustainable production.

Key words: SNP, sheep, goat, health and adaptation.
Introduction

Sheep and goats are two of the earliest animals that were domesticated by humans. Goats were derived from bezoar goats and domesticated in Western Asia between 6,000 and 7,000 B.C (Aldridge et al. 2019). The domestication of sheep occurred even earlier, between 11,000 and 9,000 BC may have derived from the European mouflon which came from the Fertile Crescent about 3,000 B.C (Aldridge et al. 2019).

Production of small ruminant animals has increased globally, mostly because of increasing demand for meat and milk (Mazinani et al. 2020). According to the FAO, the estimated world’s population will approach a 10 billion size by the year 2050. It also emphasizes that the economic status of people in developing nations will go up. Therefore, increased production is required to meet nutritional requirements for food and fiber. However, health is an important challenge in goat and sheep industry. Problems associated with internal parasites are a significant threat facing today’s small ruminant producer. Parasitic and inflammatory disease impact animal production and global food security (Charlier et al. 2014). Parasitic diseases of animals are estimated to cost tens of billions of dollars worldwide (Roeber et al. 2013). Control of pathogens such as microbes causing Mastitis, coccidia (Worku et al. 2009), and the nematode Haemonchus contortus (Gasbarre et al. 2001) is compounded by drug resistance. Because of this, much research has been dedicated to finding more sustainable and safer methods for increasing disease resistance by understanding animal genetic diversity is important in improving animal health in susceptible breeds such as the Boer (Worku et al 2009, Ekwemalor et al 2018) and in resistant breeds such as Croix hair sheep (Osei et al 2018). An approach that has gained much attention recently is in the development of innate immunity, which involves genetic changes of innate immune cells to enhance animals’ abilities to limit disease without the use of antibiotics (Worku et al 2016, Osei et al 2018, Ekwemalor et al 2018). Research has widely shown that there is significant evidence of the ability of these methods to enhance protection against disease in man and domestic animals including sheep and goats.

The innate immune system includes evolutionarily conserved “pattern recognition receptors” (PRRs) (Janeway and Medzhitov, 2002). Toll-like receptors (TLRs) and Galectins (GAL) are able to recognize bind and respond to a wide array of pathogen associated molecular patterns (PAMPS) that are essential in microbes but not in the animal (Vasta et al 2012). Toll-like receptors recognize microbial surface molecules such as lipopolysaccharide, flagellin, lipoteichoic acid, or
peptidoglycan. On the other hand, Galectins are a highly conserved family of β-galactoside binding lectins encoded by lectin, galactoside binding soluble genes (LGALS). Upon recognition of PAMPs TLR and Gal activate the innate immune system through different signaling pathways. Single Nucleotide Polymorphisms (SNP) are variants at a base pair that cause genetic variation within organisms. Genetic variation can be cost effectively evaluated using SNP technology (Edea et al. 2017; Berihulay et al. 2019). Determination of within breed or species variation between animals and adaption to an environment can aid in breed selection and sustainable approaches to controlling disease. This approach can aid in novel strategies that harness genetic variation and phytonutrients to modulate the host response and combat to pathogens.

Galectins and TLR interactions need to be better defined in livestock. Furthermore, little is known about LGALS variants in sheep and goat breeds across the world. Some sheep and goats are naturally more robust to resist parasitic infection. This is the best long-term solution and a benchmark to help improve breeds. Breeding for traits like parasitic resistance and genetic improvement of the population would really impact sheep and goat industry. For this, it is important to identify immune gene variants that modulate host-pathogen interactions and are responsible for small ruminant’s health and adaptation.

In this study, we investigated TLR and LGALS SNPs in the genome of Ethiopian sheep and Chinese goat populations adapted to different ecological conditions.

**Materials and Methods**
Sheep were sampled from five distinct sheep breeds (Pop1=16, Pop2=24, Pop3=18, Pop4=18 and Pop5=12) adapted to different geographical regions of Ethiopia. Nasal swabs were collected using Animal Swabs Collector (BlueGene Life Science, Cheongju, Korea) and Performagene LIVESTOCK’s nasal swabs (DNA Genotek, Kanata, ON, Canada) kit. DNA was isolated following manufacturer’s protocol. A total of 88 sheep were genotyped (Edea et al. 2017) with Ovine Infinium HD SNP BeadChip (600K).

A total of 308 Chinese goats (Pop6=48, Pop7=48, Pop8=48, Pop9=116, and Pop10=48) were genotyped (Berihulay et al. 2019) with Illumina Goat 50K BeadChip for galectin SNPs. An In Silico approach with Ensembl database (https://useast.ensembl.org/index.html) and SNP BeadChip (perfect matched reference SNP along with their genomic position) was utilized to identify SNPs. The minor allele frequency (MAF) of each SNPs was analyzed using plink software (Purcell et al. 2007).
Results
In this study SNP variants in TLR and LGALS were identified and their associated minor allele frequency were determined. The innate immune system relies on TLR and Gal to defend animals against pathogens by serving as PRR. Multiple TLR are found in sheep (Menzies et al. 2006) and goats (Tirumurugaan et al. 2010, Ekwemalor et al. 2018).
Our studies determined that the genes coding for TLR and GAL have SNPs that may be important in selection, breeding and management of animal health and production. Single nucleotide polymorphisms were identified in sheep and goats. Toll-like receptors were located on sheep chromosomes (Chr )2, 6, 12, 17, 19 and 26. A total of eighty-six SNPs were identified from TLR-1, -2, -3, -4, -5, -6, -9 and -10. These variations were located 3 prime UTR (1), upstream (6), downstream (14), and missense (62) regions of the TLR gene (Table 1). The single-nucleotide polymorphisms (SNPs) found within the PRRs change the structural orientation of the receptors and associated interactive features between the ligands and their corresponding receptors (Skevaki and Pararas, 2015). These topological variations influence the signaling pathways and also enable to recognize different pathogens. Polymorphic variants in TLRs may control the reaction of hosts against different pathogenic microbes, and these phenomena control the susceptibility and resistance against diseases (O’Dwyer et al., 2013). SNPs identified by targeted sequencing in TLR7 and 8 have been reported for their association with proviral load in goats (Olech et al., 2021). These variations may also affect the recognition patterns of ligands by TLRs to differentiate host resistance to pathogenic infections.

Table 1. Minor allele frequency (MAF) distribution for TLR variants of five different Ethiopian sheep populations adapted to different ecological conditions.

<table>
<thead>
<tr>
<th>Population</th>
<th>Fixed (0)</th>
<th>Rare (&gt;0 and &lt;0.05)</th>
<th>Intermediate (≥0.05 and &lt;0.10)</th>
<th>Common (≥0.10 and ≤0.50)</th>
<th>≥0.30 and ≤0.50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pop1 (16)</td>
<td>35</td>
<td>9</td>
<td>6</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>Pop2 (24)</td>
<td>32</td>
<td>11</td>
<td>3</td>
<td>36</td>
<td>21</td>
</tr>
<tr>
<td>Pop3 (18)</td>
<td>13</td>
<td>15</td>
<td>12</td>
<td>38</td>
<td>17</td>
</tr>
<tr>
<td>Pop4 (18)</td>
<td>15</td>
<td>8</td>
<td>14</td>
<td>46</td>
<td>18</td>
</tr>
<tr>
<td>Pop5 (12)</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>67</td>
<td>33</td>
</tr>
</tbody>
</table>

Pop; population.
Figure 1. Distribution of missense variants in different TLRs of sheep populations adapted to different ecological conditions.

Genes encoding Galectin (LGALS) were located on sheep Chrs 3, 7, 11, 14, 19, 21, 25 and goat Chr 18. Four (4) upstream, 10 downstream, and 4 missense gene variants were observed in sheep and goats from different breeds. A total of nineteen SNPs were identified from LGALS-1, -2, -3, -4, -7, -8, -9, -11 and -12 with a consequence of upstream, downstream, and missense gene variant (Table 2). The variation of genotypes for a given SNP was evaluated by calculating the Minor allele frequency (MAF). This is the frequency at which the second most common allele occurs in a given population. For all the TLR and LGALS variants, a highly variable MAF was observed across five sheep populations. Seven SNPS were identified in sheep LGALS 11 and 12 for all breeds tested. This approach also provides information to differentiate between common and rare variants in the population (Uzzaman et al., 2014). Breed specific TLR & LGALS gene variation was observed in sheep and goats adapted to different environments. For example, the C/T SNP on LGALS7 gene (Seq10 in Table 2) were found polymorphic for the breed in Pop2 only. The frequency of C allele (minor allele) in Pop2 is 0.25 whereas in all other populations it is zero. It means that the animals in remaining four populations don’t have C allele in that position rather they are fixed for the other (T) allele.

Table 2. Minor allele frequency (MAF) of galectin variants across five sheep populations adapted to different ecological conditions.

<table>
<thead>
<tr>
<th>Chr</th>
<th>SNP</th>
<th>A1</th>
<th>A2</th>
<th>Pop1 (16)</th>
<th>Pop2 (24)</th>
<th>Pop3 (18)</th>
<th>Po4 (18)</th>
<th>Po5 (12)</th>
<th>Galectin</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Seq1</td>
<td>C</td>
<td>T</td>
<td>0.06</td>
<td>0.33</td>
<td>0.22</td>
<td>0.39</td>
<td>0.33</td>
<td>LGALS1</td>
<td>upstream</td>
</tr>
<tr>
<td>3</td>
<td>Seq2</td>
<td>T</td>
<td>G</td>
<td>0.19</td>
<td>0.29</td>
<td>0.39</td>
<td>0.17</td>
<td>0.00</td>
<td>LGALS2</td>
<td>downstream</td>
</tr>
<tr>
<td>3</td>
<td>Seq3</td>
<td>G</td>
<td>A</td>
<td>0.31</td>
<td>0.50</td>
<td>0.44</td>
<td>0.44</td>
<td>0.33</td>
<td>LGALS2</td>
<td>upstream</td>
</tr>
</tbody>
</table>
Only 1 LGALS SNP was identified in goats using the Goat 50K BeadChip. A SNP from LGALS-4 (Seq20) was found at variable MAF level across the said goat populations (Table 3). The SNP will contribute new knowledge about goat Gal 4. Variation in Gal-4, a tandem repeat type Gal expressed in intestinal epithelial cells and secreted to the extracellular (Helwa et al., 2016). However, no TLR SNPs were detected on Goat 50K BeadChip. Of the two different genotyping platforms, the Illumina Goat 50K SNP BeadChip containing 53,347 SNPs whereas the Ovine Infinium HD SNP BeadChip (600K) higher density ovine chip can identify up to 600,000 SNPS in the sheep. With the advent of genomic technologies resulting in the availability of SNP BeadChip have identified genes that have undergone positive selection and are likely to contribute directly to phenotypic variation in several local and international breeds. It still highlights the need to investigate signatures of selection in many other sheep and goat breeds worldwide (Brito et al., 2017).

**Table 3.** Minor allele frequency (MAF) of LGALS4 variants across five goat populations adapted to different ecological conditions.

<table>
<thead>
<tr>
<th>Chr</th>
<th>SNP</th>
<th>A1</th>
<th>A2</th>
<th>Pop6 (48)</th>
<th>Pop7 (48)</th>
<th>Pop8 (48)</th>
<th>Pop9 (116)</th>
<th>Pop10 (48)</th>
<th>Galectin</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Seq</td>
<td>G</td>
<td>A</td>
<td>0.13</td>
<td>0.00</td>
<td>0.06</td>
<td>0.00</td>
<td>0.00</td>
<td>LGALS4</td>
<td>downstream</td>
</tr>
</tbody>
</table>

Seq; sequence, Pop; population.
regulation and function. These results will aid in the definition of sheep and goat TLR and Galectin phenotypes based on their genotypes and environments. This will expand capacity to characterize and measure phenomes which in turn will allow the interrogation of existing and novel genetic variation and eventually will facilitate the identification of causal genetic variation in TLR and galectins.

**Conclusion**
These studies have identified SNPS in TLR and LGALS genes. Both TLR and Gal SNPs revealed highly polymorphic variants. The observed variation may have implications for animal health adaptation and production. This new knowledge and the SNP analysis approach can increase prediction of sheep and goat innate immunity and adaptation to control diseases for sustainable production. Studies are needed on functional impacts. Future studies will include targeted sequencing of LGALS genes across different sheep and goat breeds.
References


