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A GENOME-WIDE SCAN OF POSITIVE SELECTION SIGNATURE USING OVINE INFINIUM® HD SNP BEADCHIP IN TWO ROMNEY LINES, SELECTED FOR RESISTANCE OR RESILIENCE TO NEMATODES

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SUMMARY

A study was undertaken to identify selection signatures associated with resistance to gastrointestinal nematodiasis in sheep. Ninety-three Romney sheep from two selection lines (resistant or resilient) were genotyped using the Ovine Infinium® HD SNP BeadChip, and extended haplotype homozygosity (EHH) and site-specific extended haplotype homozygosity (EHHS) analyses were undertaken. In total, 224 SNPs (147 in EHH and 77 in EHHS) were found to be significant (p <0.0001). Preliminary exploration of 10 SNPs found them to be located within two previously identified QTLs, namely LATRICH_2 and FECGEN, which were associated with nematode larval count and faecal egg count, respectively.

INTRODUCTION

Gastrointestinal nematodes are one of the most serious parasitic threats for sheep (Familton and McAnulty 1997; Perry and Randolph 1999), costing approximately $300 million annually to the New Zealand sheep industry (Rattray 2003). The current high dose usage of anthelmintics, owing to anthelmintic resistance, is not welcomed by the present global market, considering the increasing consumers' preference for organic products. Therefore, alternative anti-parasite strategies are necessary. Genetic selection is one of the most important ways in animal husbandry to improve the quality of domestic animals. Several studies have shown that resistance to nematodiasis in sheep is highly variable and heritable between individuals so that selective breeding can be an alternative choice for nematode control (Morris *et al*. 1995, 2000, 2005).

The advent of high-density single nucleotide polymorphism (SNP) microarray chips has facilitated detection of selection signatures based on patterns of linkage disequilibrium in selection lines. This is based on the assumption that the frequency of a novel mutation, that confers an advantage, will increase more rapidly than that of a neutral mutation (Sabeti *et al*. 2002). Consequently, long linkage disequilibrium (LD) blocks that incorporate genomic regions containing the causative genetic mutations could exist in populations undergoing artificial selection, given that they have been bred for insufficient generations to break the LD through recombination (Slatkin 2008). Hence, a high frequency and unusually long haplotype within a selected population could indicate the presence of a positive selection signature.

To detect these signatures, an algorithm called extended haplotype homozygosity (EHH) was initially introduced (Sabeti *et al*. 2002) which quantifies the decay of haplotype homozygosity within a population. Subsequently, another method known as the site-specific extended haplotype homozygosity (EHHS), was introduced to do the same purpose between populations (Sabeti *et al*. 2007). These methods have been successfully used to detect selection signatures in animals (McRae *et al* 2014; Somavilla *et al*. 2014; Zhang *et al*. 2012). Using the Ovine Infinium® HD SNP BeadChip the current study attempts to detect positive selection signatures in two Romney sheep lines selected for divergent approaches to coping with nematode infections, that being either resistance or resilience.

MATERIALS AND METHODS

This study was carried out following the guidelines of the 1999 New Zealand Animal Welfare Act and was approved by the Lincoln University Animal Ethics Committee (Permit Numbers: LUAEC#588). During early 2015, ear punch samples were collected into Allflex tissue sampling units (TSU), using an Allflex NZ tissue sampling applicator (TSU Applicator – 22134), from 93 Romney sheep belonging to two selection lines (nematode resistant, n = 42, and nematode resilient, n = 51), currently being maintained at Lincoln University, Lincoln, New Zealand. Details regarding the selection lines were described elsewhere (Morris *et al*. 2000). Sheep in the two lines were selectively bred based on faecal egg count (FEC), for at least 24 years (1985-2009) and since then have been randomly bred within each line. The tissue samples were submitted to AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand, for DNA extraction and SNP genotyping using the Ovine Infinium® HD SNP BeadChip.

The original SNP data (idat files) were converted into to PLINK format (PED/MAP) in GenomeStudio® (Illumina, San Diego CA, USA). Quality control was performed using PLINK_v1.9 (Chang *et al*. 2015; Purcell *et al*. 2007). A within individual call rate threshold of 99% was applied and SNPs with a call rate <95%, or a minor allele frequency <1%, or a *p* value of $<10^{-6}$ for Hardy-Weinberg equilibrium were excluded. After quality control a total of 463,392 SNPs, located on the 26 autosomes in all 93 sampled individuals were retained for further analysis.

The SNP data was reformatted in PLINK and inputted into fastPHASE_v1.4 (Scheet & Stephens 2006) in order to reconstruct the haplotypes for each autosome, using the default parameters. The resultant haplotype data (phased data) was used to detect positive selection signatures by calculating the allele-specific extended haplotype homozygosity (EHH) within populations as well as the site-specific extended haplotype homozygosity (EHHS) between populations, using an R package, REHH 2.0 (Gautier *et al*. 2017). For EHH, the test statistic was iHS (Gautier & Naves 2011), standardized ratio of the integrated allele-specific EHH (iHH), while for EHHS, two separate test statistics were employed – xp-EHH (Sabeti *et al*. 2007) and Rsb (Tang *et al*. 2007). Significance of detected signatures of selection was determined based on the *p* values for iHS, xp-EHH and Rsb.

RESULTS AND DISCUSSION

The purpose of this study was to identify SNPs or genes exhibiting positive selection from the pressure of nematodes. SNP genotypes pertaining to 463,392 markers covering the 26 autosomes were used. Since the two selection lines investigated in the study were selectively bred for at least 24 years (1985-2009) based on faecal egg count (FEC) using best linear unbiased prediction (BLUP) techniques and since then randomly bred within each line, these populations are suited to investigations of selection signatures of long time breeding associated with resistance and resilience.

EHH testing revealed 62 and 85 SNPs to exhibit positive selection signatures ($p<0.0001$) in the nematode resistant and resilient groups, respectively. None of the identified SNPs were shared between the two lines, indicating genetic differences (possibly due to selection pressure) between these two populations. An iHS plot for OAR2 for the two lines, revealing the differences between the two populations is shown in Figure 1. EHHS testing was also performed to detect positive selection signatures between the two populations. Two different algorithms, XP-EHH and Rsb were used. A total of 39 and 48 SNPs were detected exhibiting positive selection signatures, in the two algorithms, respectively. None of these SNPs were common to those detected in the within-line EHH analysis. Figure 2 depicts the results for the Rsb and XP-EHH algorithms with respect to markers on chromosome 13. So far, there is no demonstrated advantage of one over the other, and the results from these two algorithms can be slightly different (Gautier *et al*. 2017). Therefore, the SNPs shared by two algorithms should be more reliable. Ten SNPs (Table 1) were shared between the two algorithms and those were found to be located in regions of known significance and hence, can be considered as highly confident SNPs associated with nematode resistance and resilience in

sheep. A QTL search (http://www.animalgenome.org/QTLdb/sheep) showed the SNP oar3_OAR11_48327544 to be located within the reported QTL:12901, which has been demonstrated to be associated with larva count (Crawford *et al*. 2006). The remaining nine SNPs were all located within a small region overlapping the gene Protein Tyrosine Phosphatase, Receptor Type T (*PTPRT*) on chromosome 13 (Oar_v4.0). *PTPRT* is a protein coding gene and GO annotations show that this gene is related to *phosphatase activity* and *beta-catenin binding*, possibly indicating that this gene could have a function in resistance to nematodes. Further, these SNPs are harboured within the previously identified QTL:16027, which has been associated with faecal egg counts (Sonstegard *et al*. 2012).

A previous study (McRae *et al.* 2014) performed on Romney and Perendale sheep, that used F_{ST} and Peddrift to detect differentiation between nematode resistant and susceptible lines, identified sixteen significant regions, which included candidate genes involved in chitinase activity and the cytokine response. The samples from nematode resistant sheep obtained for the current study came from the same line as that from McRae *et al.* (2014), but were from a different generation. In the current study, apart from the 10 significant SNPs detected by both the Rsb and XP-EHH algorithms, there were 77 other significant SNPs detected by one or either of the algorithms. These 77, together with the 147 SNPs (62 and 85 in the resistant and resilient lines, respectively) detected in the EHH analysis will be explored in detail to find out if they are located in or near to gene regions of immunological significance.

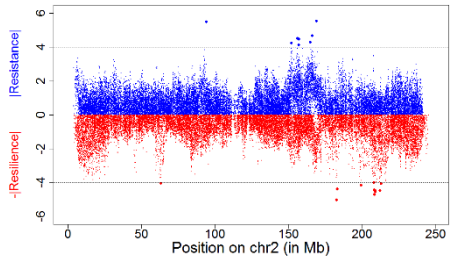

Figure 1 iHS difference for markers on chromosome 2 between lines of Romney sheep selected for either resistance or resilience to gastro-intestinal nematodes

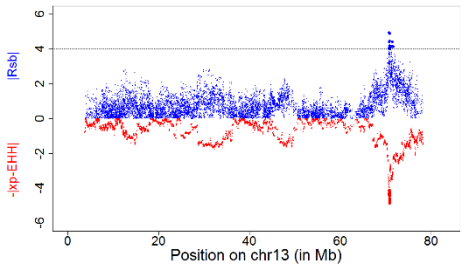

Figure 2 REHH difference between XP-EHH or Rsb methods for markers on chromosome 13

CONCLUSION

This study provided a genome-wide map of positive selection signatures in two Romney sheep lines selected for FEC. Several significant SNPs were identified and preliminary analysis of ten of the identified SNPs revealed that they were located within two previously detected QTLs associated with gastrointestinal nematodiasis in sheep. The significance of the remaining SNPs is currently being explored.

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Table 1. SNP markers detected by both the EHHS algorithms, XP-EHH and Rsb, to suggest evidence of positive selection in lines of Romney sheep selected for either resistance or resilience to gastro-intestinal nematodes

SNP	Chr	Position	Gene		QTL ID	
oar3_OAR11_48327544	11	48327544	none	LATRICH_2	QTL:12901	larva count
oar3_OAR13_70810243	13	70810243	PTPRT	FECGEN	QTL:16027	FEC
oar3_OAR13_70820259	13	70820259	PTPRT	FECGEN	QTL:16028	FEC
oar3_OAR13_70853062	13	70853062	PTPRT	FECGEN	QTL:16029	FEC
oar3_OAR13_70853714	13	70853714	PTPRT	FECGEN	QTL:16030	FEC
oar3_OAR13_70870621	13	70870621	PTPRT	FECGEN	QTL:16031	FEC
oar3_OAR13_70876794	13	70876794	PTPRT	FECGEN	QTL:16032	FEC
oar3_OAR13_70887333	13	70887333	PTPRT	FECGEN	QTL:16033	FEC
oar3_OAR13_70891326	13	70891326	PTPRT	FECGEN	QTL:16034	FEC
oar3_OAR13_70896117	13	70896117	PTPRT	FECGEN	QTL:16035	FEC

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