TARGETED MAPPING OF QTL ON CHROMOSOMES 1 AND 3 FOR PARASITE RESISTANCE IN SHEEP

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SUMMARY

The aim of this project is to identify ovine quantitative trait loci (QTL) affecting resistance and susceptibility to internal parasites. For this study, we focused on previously identified QTL regions on OAR1 and OAR3 by adding an extra 12 markers to the 15 formerly mapped on OAR1, while 14 were added to OAR3, making a total of 28. Significant QTL for FEC were identified on both chromosomes, at 188 and 184 cM respectively. Further reinforcing these findings, a meta-analysis of published QTL supported these locations. The meta-analysis was used to determine the density and location of SNPs used in the development of a 1536 custom SNP golden gate assay, for a combined LA/LD analysis.

INTRODUCTION

The cost of gastro-intestinal nematode (GIN) infection to the Australian sheep industry exceeds $200 million pa. Control of GIN has primarily focused on the use of anthelmintic drenches. However, the evolution of drench-resistant parasites, coupled with increasing consumer concern over chemical use in agricultural industries, has instigated research into alternative forms of parasite control. Certain breeds of sheep are naturally resistant to GIN (Vanimisetti *et al.* 2004), and significant genetic variation within breeds strongly indicates that genetic factors play a substantial role in host resistance to parasites. Characterization of genes responsible for variation in host resistance has been restricted to QTL and candidate gene analyses. However, few studies have identified causative mutations, primarily due to a lack of effort in fine mapping QTL. Here we present the results of progress towards fine mapping of two QTL previously identified from a whole genome scan.

MATERIALS AND METHODS

Background. Ten half sibling families (n = 694) were created by mating Indonesian Thin Tail (ITT) cross Merino (M) F1 sires to M, ITT and F1 ewes over 4 seasons. The sheep were raised and kept indoors on slatted floors and were subjected to two sequential 10 week challenges with thrice weekly administration of 2000 L3 *H. contortus* larvae for the first 3 weeks of each challenge period. Phenotypic characteristics measured were faecal egg counts (FEC), live weights (Wt) and packed cell volumes (PCV), which were recorded on the first day of the challenge and at fortnightly intervals thereafter for 10 weeks after initial challenge.

QTL analysis. FEC data was normalised using the accepted method of cube root transformation as log transformation over corrected some traits. All phenotypes were adjusted for fixed effects. QTL analysis was performed using QTLExpress (Seaton *et al.* 2002) with 5000 permutations and bootstraps for establishment of significance thresholds and QTL locations. As preliminary results from the whole genome screen (unpublished results) indicated the presence of QTL on OAR1 and OAR3, an additional 12 and 14 markers were added to those chromosomes respectively.

Meta-analysis and SNP selection. A meta-analysis was performed as described in Khatkar *et al.* (2004) to determine consensus locations for QTL on OAR1 and 3 for parasite traits. All papers that reported QTL on OAR 1 or 3 were included. The QTL location, confidence interval (CI) and a pre-assigned score according to the importance of the QTL were used to calculate a sum of scores at each putative QTL position along the chromosomes. These meta-scores were then used to select SNPs to be incorporated on a custom array designed to allow fine mapping of parasite traits in both ours and other populations, with the eventual aim of developing a diagnostic set of markers for use in breeding programs. The 1536 SNPs that could fit onto the array were distributed across the areas of interest according to the strength of the QTL score in that region, and were selected from the Virtual Sheep Genome resource (Dalrymple *et al.* 2007). Selection criteria were Illumina Score (to assess the likely success of the oligo design) >0.8, position, and MAF > 0.3. Genotyping is underway but was not completed at the time of publication.

RESULTS AND DISCUSSION

QTL Mapping. Following the addition of 12 markers to OAR 1 (27 markers in total) and 14 to OAR3 (n = 28), the information content increased markedly (Figure 1).

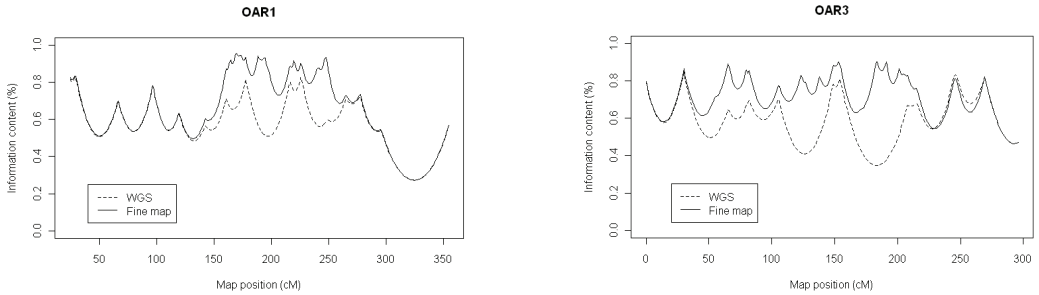

Figure 1: Information content along OAR1 and 3 before after fine mapping. The dotted line shows information content of the original scan, while the solid line shows the content after the addition of fine mapping markers.

Previously identified QTL for Wt change on OAR1 and FEC2 on OAR3 (data not shown) were not significant following the genotyping of extra markers. However, on each chromosome, one QTL for FEC during the first challenge remained significant (Table 1). The additional markers improved the F statistic from 2.95 (P<0.05 chromosome wide) to 4.37 (P<0.05 genome wide) for the QTL on OAR1, and from 1.64 (not significant) to 3.03 (P<0.05, genome wide) on OAR3. However, the confidence intervals remained large (Table 1), as illustrated by bootstrap analyses showing possible distribution of QTL locations in Figure 2.

Table 1: Significant QTL detected on OAR1 and 3. The framework map positions were taken from the sheep linkage map v4.7 (http://rubens.its.unimelb.edu.au/~jillm/jill.htm

Trait	QTL position (cM)	F	LR	Significance thresholds				95% CI (cM)
				Chromosome wide		Genome wide		
				0.05	0.01	0.05	0.01	
OAR 1 FEC1	188	4.37	42.55	2.945	3.616	3.770	4.660	68-352
OAR 3 FEC1	184	3.03	29.83	2.971	3.606	3.770	4.660	0-252

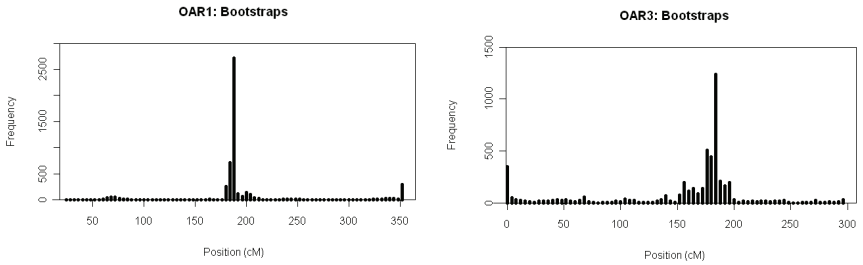

Figure 2: Distribution of possible QTL locations (5000 bootstraps)

Meta-analysis and SNP selection. The meta-analysis included 3 peer reviewed whole genome QTL studies (Crawford *et al.* 2006; Davies *et al.* 2006; Beraldi *et al.* 2007) and 8 unpublished reports, including milestone reports from collaborators and an update of the Beh (2002) project. The analysis indicated there may be a number of small QTL on each chromosome, instead of revealing strong evidence of a single QTL. The meta-scores were used to design the custom SNP array, with 1534 SNPs selected on OAR1 and 3 using the densities suggested in Table 1, while one SNP each from OARX and Y were included for quality control purposes.

Table 2: Suggested SNP positions and densities for custom parasite SNP array.

Area (cM)	Priority	SNP Density (/cM)	Flanking markers	Interval Length (cM)	SNPs
OAR1					
75–150	Med	3	OARCP93/UMJM36 - APPO10/BMS574	75	225
150–180	High	10	APPO10/BMS574 - MNS94/CSSM4	30	300
190–250	Med	3	MNS94/CSSM4 - MCM130/TEXAN6	60	180
300–325	Med	3	UWCA46/KD721 - MCM357/EPCDV13	25	75
Total				190	780
OAR3					
25–50	Med	3	DIK5391/ILSTS28 - BM746/BM1861	25	75
130–190	Med	3	BM2818/BMS1953 -UCD52/BL4	60	180
190–230	High	10	UCD52/BL4 - TEXAN15/PTHLH	40	400
230–260	Med	3	TEXAN15/PTHLH - ILSTS34/UCD14	30	90
Total				155	745
Overall				345	1525

Following the placement of additional markers on OAR1, the significant QTL mapped to a different position to previously published FEC QTL, found at around 270 - 340 cM and 79 cM respectively (Beh *et al.* 2002; Beraldi *et al.* 2007). However, the large CI spans the locations of all QTL identified in these studies so it is possible that the QTL detected by us and others in different positions may actually be caused by a common underlying genetic factor. A similar result was seen on OAR3, where previous studies have detected significant QTL for FEC and immune responses (Beh *et al.* 2002; Davies *et al.* 2006) as well as numerous suggestive QTL at positions between 0 - 35, 95 - 180 cM, and 200 - 240 cM (Crawford *et al.* 2006; Beraldi *et al.* 2007).

Due to the ambiguity of the QTL positions as indicated by large CI, which in both cases cover most of the chromosome, it was thought that performing the meta-analysis would be useful to determine the most suitable regions to add more SNP markers for fine mapping. However, on both chromosomes, the meta-analysis did not indicate the presence of a single QTL. It was thus decided to place SNPs over a larger area of the chromosome than just those regions surrounding the indicated QTL position, with increasing SNP density in the priority area as determined by the

combined weighted score from the meta-analysis. On OAR3, the priority area indicated by the meta-analysis was slightly downstream of the most likely position of our QTL (184 cM), and included the region surrounding the interferon-γ gene. Although a marker within interferon-γ (205.5 cM) has previously been implicated as contributing to variation in host resistance to parasites (Coltman *et al.* 2001, Paterson *et al.* 2001), other studies have failed to replicate these results, and we found no evidence to support this within our flock (Raadsma *et al.* 2008). The addition of SNP markers spread over a large area on both chromosomes will allow us to perform more rigorous LA/LD analyses. This method uses simultaneous multi-trait analyses and combines linkage analysis (LA) information with linkage disequilibrium (LD) information, thus preventing spurious associations, improving power and precision, and reducing confidence intervals, resulting in more specific QTL locations for the identification of causative polymorphisms (Meuwissen and Goddard, 2004).

CONCLUSION

We have mapped two QTL for FEC on OAR1 and 3, and performed a meta-analysis of other studies, published and otherwise, combining the information for targeted fine mapping using a custom designed 1536 SNP array. Fine mapping using LA/LD analyses methodologies to narrow down the region is critical before systematic positional candidate gene searches can begin. In the future we hope to identify and characterize genetic markers for parasite traits in sheep, allowing farmers the option to selectively breed their flocks for resistance and resilience to gastro-intestinal nematodes.

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