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A GENOME SCAN FOR QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO THE GASTROINTESTINAL PARASITE *HAEMONCHUS CONTORTUS* IN SHEEP

K. Marshall[1,2], J. H. J. van der Werf[1,2], J.F. Maddox[3], H-U. Graser[4], Y. Zhang[4], S. W. Walkden-Brown[1] and L. Kahn[1].

[1]School of Rural Science and Agriculture, University of New England, Armidale, NSW, 2350
[2]The Institute of Genetics and Bioinformatics, University of New England, Armidale, NSW, 2350
[3]Centre for Animal Biotechnology, School of Vet Science, University of Melbourne, Victoria, 3010
[4]Animal Genetics and Breeding Unit, University of New England, Armidale, NSW, 2350

SUMMARY

This paper presents some results from a genome scan for QTL affecting resistance to the gastrointestinal nematode *Haemonchus contortus*. The mapping flock comprised 571 sheep from four half-sib families, sired by individuals from the *Golden Ram* flock. The traits analysed were faecal egg counts after two artificial challenges at 6 and 13 months of age, termed FEC12 and FEC34 respectively. The correlation between these traits was low at 0.21. Animals were selectively genotyped for a total of 223 markers, and interval mapping performed on a within and across-family basis. For the within-family analysis 11 QTL (as chromosomal / family combinations) were significant at the 1% chromosome-wide (CW) level, with a further 18 significant at the 5% CW level. The allelic substitution effect (α) ranged from 0.19 to 0.50 phenotypic standard deviation units (σ_P). QTL significant at the 1%CW level were located on 9 different chromosomes, with only one of these regions significant for both FEC12 and FEC34. For the across-family analysis two chromosomal regions were significant at the 5%CW level, one each for FEC12 and FEC34.

Keywords: QTL, mapping, linkage analysis, sheep, *Haemonchus contortus*

INTRODUCTION

Gastrointestinal (GI) nematode infection is a major health and economic problem of the Australian sheep industry. Parasite resistance to anthelmintics is high, genetic progress towards host resistance in industry is slow, and there is limited knowledge of the underlying trait biology. As such, identification of QTL conferring host resistance is considered a priority. This paper presents some results from a genome scan for QTL affecting resistance to the GI nematode *Haemonchus contortus* in sheep. Sires used in the mapping experiment were from the *Golden Ram* (*GR*) merino flock of the University of New England. Previous segregation analysis studies in the GR flock indicated the presence of a major gene of considerable effect (allele substitution effect (α) > 1 phenotypic standard deviation (σ_P)) (Meszaros *et al*. 1999).

MATERIALS AND METHODS

Four half-sib families were generated from *GR* flock sires with high (>80%) probabilities of being heterozygous for a major gene according to segregation analysis. The *GR* sires were mated to unrelated merino ewes, with one sire ('996110') additional mated to a small group of *GR* ewes. Family sizes were 219 (or 186 excluding *GR* ewes), 164, 102 and 86 for sires '996110', '939119', '996137' and '996169', respectively.

Faecal egg counts (FECs) were collected after two artificial challenges with *H. contortus*. The first challenge was performed when animals were about 6 months of age, with FECs collected 28 and 35 days post-infection. The second challenge was performed when animals were about 13 months of age, with FECs collected 25 and 31 days post-infection. Individual FECs were cube-root transformed, averaged within a challenge, and corrected for the fixed effects of sex, birth-type, and age. The resultant values were given the trait names FEC12 and FEC34 for the first and second challenges respectively. Both FEC12 and FEC34 had reasonably normal distributions, with means and standard deviations (in brackets) of 18.08 (6.95) and 8.52 (5.34) respectively. It is interesting to note that the Spearman rank correlation between FEC12 and FEC34 was low at 0.21, although the correlations between FECs within a challenge were high at >0.70.

Individuals from the top and bottom 25% of the FEC12 distribution on a within-family basis were selectively genotyped, except for the small group of progeny from the *GR* ewes which were all genotyped. In total 223 markers were genotyped, with between 140 and 177 informative markers per family. The markers covered all autosomes and the X-chromosome pseudoautosomal region, with an average spacing of around 20cM. Linkage maps were constructed using cri-map.

QTL mapping was performed via interval mapping using logistic regression (LR) on a within-family basis, as well as via maximum likelihood (ML) across families. The likelihood ratio test (LRT) was used as the test statistic, and empirical threshold levels were determined at the point-wide (PW) and chromosome-wide (CW) level via permutation testing using 500-1000 permutations. The LR and ML methodology is described in Henshall & Goddard (1999) and Kerr *et al*. (2005) respectively.

RESULTS AND DISCUSSION

Unfortunately chromosomal locations cannot be disclosed at this stage, due to restrictions imposed by the funding body. Table 1, however, lists the significant regions (using code) and the associated QTL effects and LRTs for the within and across-family analysis. Confidence intervals around the QTL positions were typically 30-50 cM.

For the within-family analysis, 2 and 9 QTL (as chromosomal / family combinations) were significant at the 1%CW level for the traits FEC12 and FEC34 respectively. The higher number of putative QTL for FEC34 was unexpected, given animals were selectively genotyped on the basis of FEC12. For both traits an additional 9 QTL were significant at the 5% CW level. Over both traits, the theoretical false discovery rate (FDR) at the 1% and 5% levels were 0.21 and 0.39, respectively.

QTL significant at the 1%CW level were located on 9 chromosomal regions, with QTL significant at the 5%CW level located on an additional 16 chromosomal regions. Most commonly, QTL for FEC12 and FEC34 were located at different positions, consistent with trait correlation of 0.21. Three regions did, however, appear to harbor QTL for both FEC12 and FEC34 (coded in Table 1 as A1, H1, and I1) with one of these regions (A1) obtaining 1%CW significance for both traits.

All families were segregating for at least one QTL significant at the 5%CW level. Interestingly, the number of QTL per family was highest for sire 996137 (14 QTL detected), which had one of the smaller progeny groups, and lowest for sire 939119 (2 QTL detected), which had one of the larger progeny groups. There was also a tendency for the smaller families to segregate for the QTL of the

Table 1. Quantitative trait loci for faecal egg counts from within and across-family analysis

Trait	Family	Location code[1]	α[2]	LRT[3]	5%CW LRT[3]	1%CW LRT[3]	Sig[4]
Within-family analysis							
FEC12	996137	A1	0.38	10.95	5.72	6.98	*
FEC34	996137	A1	0.48	8.76	5.37	7.48	*
FEC34	996110	B1	0.23	5.51	2.88	3.60	*
FEC34	996110	C1	0.31	10.98	5.11	7.67	*
FEC12	996137	C2	0.26	4.55	4.54	6.41	
FEC12	996110[5]	C3	0.22	5.83	4.83	7.10	
FEC34	996137	D1	0.45	7.60	3.72	5.79	*
FEC12	996137	D2	0.28	5.02	3.11	5.58	
FEC34	939119	E1	0.35	10.66	6.65	8.68	*
FEC34	996137	E1	0.50	9.45	5.85	8.26	*
FEC12	996110	E2	0.23	8.28	7.12	9.73	
FEC34	996110	F1	0.21	4.95	3.40	4.78	*
FEC34	996137	G1	0.38	4.89	2.41	4.75	*
FEC12	996137	H1	0.29	5.73	4.15	7.70	
FEC34	996137	H1	0.41	5.92	3.80	5.78	*
FEC12	996137	I1	0.29	5.43	4.35	5.42	*
FEC34	996169	I1	0.40	5.21	3.51	5.39	
FEC34	996137	J1	0.42	6.32	3.57	6.57	
FEC34	996137	K1	0.41	6.16	3.98	6.47	
FEC34	996169	L1	0.37	4.19	2.58	4.50	
FEC12	996169	M1	0.29	5.39	4.48	5.81	
FEC34	996110[5]	N1	0.21	5.10	3.79	5.80	
FEC34	996110	O1	0.18	4.24	2.73	4.97	
FEC12	939119	P1	0.24	5.89	4.57	7.34	
FEC34	996137	Q1	0.37	4.45	3.42	5.62	
FEC12	996110[5]	R1	0.19	4.26	3.88	5.82	
FEC34	996137	R2	0.37	4.70	3.97	6.63	
FEC34	996169	S1	0.40	5.11	4.94	7.78	
FEC12	996110	S2	0.21	5.55	4.62	8.47	
Across-family analysis							
FEC34	-	C1	0.38	10.10	6.8	11.16	
FEC12	-	T1	0.26	9.36	7.3	10.73	

[1] Letters in the location code indicate chromosomes, whilst numbers indicate regions on the chromosome. For example C1, C2 and C3 represent three different regions on chromosome 'C'.
[2] α is allelic substitution effect in phenotypic standard deviation units.
[3] Likelihood ratio tests (LRT) at the most likely position, and the 5% and 1% chromosome-wide thresholds (5%CW LRT and 1%CW LRT respectively).
[4] An * indicates 1%CW significance, all others obtained 5%CW significance.
[5] For analysis excluding the small group (n=33) of *GR* ewe progeny.

larger effect. This may indicate a greater number of false positives for the smaller families, although this cannot be confirmed without further studies. The allele substitution effect (α) ranged from 0.19 to 0.38 σ_P for FEC12, and from 0.21 to 0.50 σ_P for FEC34. This is considerably lower than that estimated by segregation analysis (which was 1 to 1.5 σ_P). Investigations for the discrepancy between segregation and mapping analysis results are underway.

Only two chromosomal regions from across-family analyses were significant at the 5% CW level. These were region T1 for FEC12 (α=0.26 σ_P), and region C1 for FEC34 (α=0.35 σ_P). For region T1 the across-family analysis was significant due to an accumulation of information over families: under the within-family analysis 3 families had QTL significant at the 5% PW level in this region. For region C1 the significant across-family result was due to a very significant QTL in one family only. A further region, E1, contained QTL significant at the 1%CW level for FEC34 in two families under single family analysis. Under across-family analysis this was significant at the 1%PW level.

Literature regarding QTL for resistance to GI nematodes is limited. Published genome scans disclosing chromosomal locations include the studies of Beh *et al.* (2002) for resistance to *Trichostrongylus colubriformus* in sheep, and Iraqi *et al.* (2003) who used a mouse model. Other studies have focused on particular chromosomes, such as 1, 3 and 20 (reviewed in Dominik 2005). The finest mapped QTL to date is a 5cM region on *Chr* 3 (near INF-γ), reported by Paterson *et al.* (2001). Groups (in addition to ourselves) previously or currently undertaking mapping experiments include CSIRO (Australia), the University of Sydney (Australia), AgResearch (New Zealand), Louisiana State University (USA), and ILRI (Kenya). Given the inherent difficulties of mapping QTL for disease resistance traits, a meta-analysis across all studies would clearly be beneficial in terms of identifying regions significant across different host immune states and parasite types.

Concluding comments. The results presented here are promising, with numerous potential QTL of moderate effect (up to 0.5 σ_P) identified. Following on from this work several regions have been selected for confirmatory studies and possible fine-mapping.

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