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A SNP ASSOCIATION STUDY VERIFIES A MAJOR LOCUS FOR FIBRE DIAMETER RELATED TRAITS ON CHROMOSOME 25 IN SHEEP

H.W. Raadsma[1], E. Jonas[1,2] and P.C. Thomson[1]

[1] ReproGen, Faculty of Veterinary Science, The University of Sydney, Camden, NSW, 2570
[2] Swedish University of Agricultural Sciences – Department of Animal Breeding and Genetics, 750 07 Uppsala, Sweden

SUMMARY

The present paper reports on the fine mapping of a previously identified quantitative trait locus (QTL) for fibre diameter and fibre diameter variability on chromosome 25 in sheep. A medium-density SNP panel was used to narrow down a major linked region using 319 animals from an Awassi-Merino backcross population. We could narrow down the QTL region of interest to 5000 kbp (3000 to 6000 kbp) on chromosome 25. Strong pleiotrophic effects were previously seen for this QTL as linkage had been identified for mean fibre diameter, fibre diameter variability, proportion of coarse fibres and comfort factor. Histological examination of animals with extreme fibre diameter characteristics showed strong effects for mean diameter of primary follicles, and a much higher ratio of secondary to primary follicles (S:P ratio) for animals inheriting the fineness QTL allele. A possible mode of action of the QTL on secondary follicle branching has been proposed. A strong positional candidate gene has been suggested in a companion paper (Jonas *et al.* 2013-these proceedings), however, further investigations are needed for a better understanding of the underlying causal mutations.

INTRODUCTION

Fibre production is typical of sheep, which shows marked diversity across and within breeds. Across breeds a range of fleece types can be seen including coarse from short-hair types, medulated carpet-wool types, through to ultrafine apparel wool types. In some cases selection for desired fleece characteristics has come under intense selection as for example in specialised wool breeds such as Merino. The development of efficient breeding programs relies on the identification of appropriate traits for improvement; a precise knowledge of the genetic parameters and evaluating selection strategies. Despite our broad knowledge that fleece characteristics will respond strongly to directional selection (Atkins 1997), relatively little is known about underlying genetic architecture of genes contributing to such variation within and between breeds for all major fleece characteristics. Recent developments in molecular genetics have broadened our understanding of the genetic architecture of polygenic complex-traits under selection. A better understanding of the effects and magnitudes of allelic differences that influence these traits may significantly enhance overall response of selection by improved management of antagonistic and pleiotrophic effects. Fibre diameter is one such trait of major interest, yet limited QTL detection studies have been conducted for this trait (Purvis and Franklin 2004). The advent of high-density genotyping platforms for Single Nucleotide Polymorphisms (SNP) has opened the possibility to undertake high resolution mapping approaches exploiting variation between and within breeds.

In this paper, we report a high-density SNP marker association analysis using a paternal half-sib design within an Awassi × Merino resource population. Animals were genotyped using the ovine 50k SNP array to fine-map a QTL region with impact on fibre diameter reported previously (Raadsma et al unpublished).

MATERIALS AND METHODS

Animals and Phenotypes. A resource population derived from crosses between fat-tail Awassi (A) and small-framed Merino (M) sheep was established to exploit the extreme differences between these two types in a range of production characteristics (Raadsma *et al.* 2009). In the association study reported here, data from 319 Merino backcross ((Awassi x Merino) x Merino) progeny of the first F_1 sire were analysed in detail. All lambs were shorn at 16 month of age and wool quantity was measured from mid-side samples at week 75 by the Riverina Wool Testers in Wagga Wagga, Australia. Among many other traits, mean fibre diameter, variability in fibre diameter, percentage coarse fibres, prickle, and follicle curvature were recorded from animals of

this population. For the study presented here, only fibre diameter data was used for the association analysis. QTL transmission probabilities were calculated for all animals inheriting either the paternal copy of the "coarse fibre allele-Q" or the "fine fibre allele-q". From each population, 20 animals with the most extreme fibre diameter (highest mean fibre diameter in case of Q and lowest mean fibre diameter in case of q) were selected for histological examination. Histological measurements included mean fibre diameter and fibre diameter variability of primary (P) and secondary (S) follicles, total follicle density, P and S follicle density, and S:P ratio.

Genotyping. All 319 backcross animals and the F_1 sire were genotyped using the ovine 50kb SNP array (http://www.sheephapmap.org). The predicted map positions of each SNP were used to select a subset of 757 SNP on chromosome 25. Genotypes with a minor allele frequency < 0.05 and a call rate < 0.95 were excluded from the final analysis. PLINK (Purcell *et al.* 2007) was used to check the gender and pedigree information and estimate the similarities between sire and offspring. Inheritance of the SNPs was checked according to pedigree expectation and corrected using code written in 'R' (R Core Team 2012).

Association analysis. Two slightly different models were applied to the data for the association analysis. The 'identical-by-state' matrix (IBS) was obtained in PLINK (Purcell *et al.* 2007) and the factor of similarity between sire and each offspring from the IBS was used for further analysis in 'R'. The following model was first fitted to the data of chromosome 25:

$$FT_j = \beta_0 + \beta_1 SSim_j + SNP_i + \varepsilon_{ij} \qquad [1]$$

where FT_j = Fibre trait of offspring *j*; $SSim_j$ = Similarity between offspring *j* and the sire derived from the IBS matrix; SNP_i = *i*-th SNP (i = 1 to 757 SNPs used in the study); and ε_{ij} = residual random error term.

Additionally a model was used following the approach previously applied for QTL mapping. Similar to the QTL model in QTL-MLE used to identify linkage regions (Raadsma *et al.* 2009), the probability of allele '1' derived from the dam was deducted from the dataset and used as a fixed effect for the whole-genome association analysis using an alternative linear model in 'R'.

$$FT_j = \beta_0 + \beta_1 PDam_i + \varepsilon_i \qquad [2]$$

where FT_j = Fibre diameter of offspring *j*; $PDam_i$ = probability of allele 1 at SNP *i* transmitted from Dam; and ε_i = residual random error term.

Using these two slightly different models aimed to exploit some background information provided through the sire and or dam side. Firstly using additional information from the sire (Awassi x Merino) which aimed to utilize allelic information on the paternal side transmitted through the F1 sire. And secondly by using additional information on inherited maternal Merino alleles (1 or 2 alleles) more explicitly as fitted in model 2.

RESULTS AND DISCUSSION

Phenotypic data. The average fibre diameter was 24 μm, which equates to medium-strong wool, with values between 18 μm (equivalent to super-fine Merino) and 30 μm (equivalent to strong-wool Merino or crossbred wool type) (Atkins 1997).

Association analysis. Using the same resource flock, we have previously identified a number of QTL for various fleece quality and quantity traits using a genome wide linkage analysis with microsatellite markers as detailed by Raadsma et al (2009), among which a region on chromosome 25 stood out revealing one or more highly significant linkage regions (Raadsma et al unpublished). Details of initial QTL probability using microsatellite linkage analyses have been shown in Figure 1 for reference. However as no candidate gene had been described previously within the identified region in sheep and/ or comparative chromosomes in other species, further studies had been suggested to narrow down the region of interest. The study here aimed therefore to fine-map and to verify the previously identified QTL on chromosome 25. Models considering either Awassi or Merino influence on offspring genotypes showed significant association for fibre diameter in the region around 5000 kbp (3000 to 6000 kbp) on chromosome 25. Results of association analysis of with fibre diameter are shown in Figure 1 for each SNP positioned along the chromosome. The

results of both models were in good agreement. Results also verified the previous linkage region and could narrow down the significant associated region.

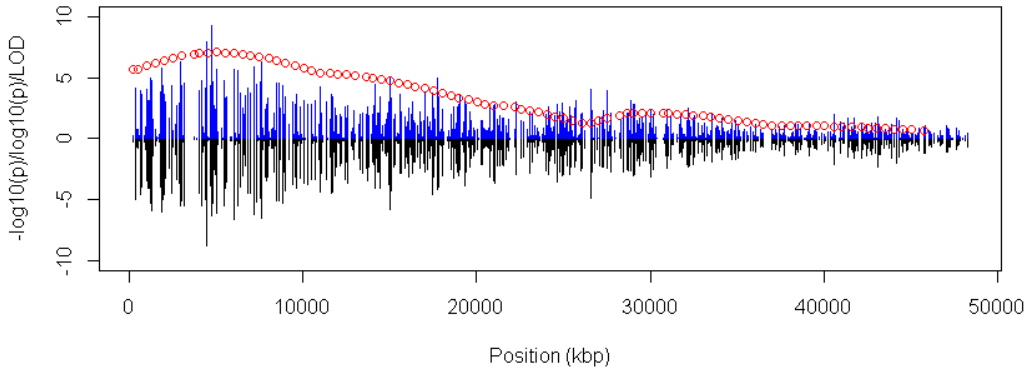

Figure 1. Results of the association analysis for fibre diameter using model [1] (above x-axis) and model [2] (under x-axis). Indicated with red dots are the LOD scores from the QTL study for fibre diameter from an unpublished linkage analysis using the same resource flock and data set

As reviewed by Purvis and Franklin (2004) only a limited number of QTL and GWAS studies have been conducted on fibre traits. QTL were identified for fibre diameter on chromosome 25 in a in a synthetic breed Merino based INRA401 (Ponz *et al.* 2001), a backcross Sarda x Lacaune sheep resource population (Allain et al. 2006), fine and superfine Merino sheep (Bidinost *et al.* 2008). This study confirms the importance of a major gene for fibre diameter characteristics and fleece quality. Within the positional candidate region, a positional candidate gene could be identified, results are shown elsewhere (Jonas *et al.* 2013).

Table 2. Contrasts of skin follicle characteristics in animals inheriting the paternal coarse-fibre (Q n=11) vs fine fibre (q n=9) QTL on OAR 25 from an Awassi-Merino to Merino backcross QTL mapping population

trait	Paternal coarse wool allele-Q		Paternal fine wool allele-q		% change
	mean	sd	mean	sd	(Qvs q)/q
Follicle density (n/mm**2)	54	8.4	73	13	+36
Density primary follicle(n/mm**2)	3.9	0.64	4.2	0.92	+7
Density secondary follicle(n/mm**2)	50	8.4	69	13	+38
Ratio S:P follicle	13	3.2	17	3.6	+30
Mean FD all follicles (um)	28	3.0	23	1.8	-19
SD FD all follicles (um)	8.0	0.7	4.0	0.8	-22
Mean FD primary follicles (um)	49	2.8	26	3.4	-46
SD FD primary follicles (um)	10	1.5	4.4	0.7	-56
Mean FD secondary follicles (um)	27	3.0	23	1.8	-15
SD FD secondary follicles (um)	5.1	0.72	4.0	0.81	-22

The mode of action of the QTL is likely to be through regulation of secondary follicle density during embryonic development given the large difference in secondary follicle density and ratio between secondary and primary skin follicles in animals with contrasting (“coarse fibre allele-Q” versus “fine fibre allele-q”) QTL alleles. Follicle branching is often thought to be a major characteristics of fine wool Merino sheep resulting in high secondary follicle populations in the skin. The main influence for differences of fibre diameter in the sheep used here was through a much higher S:P ratio in the animals inheriting the allele for fineness, suggesting a gene linked to

follicle development and possibly the branching of secondary follicles (Table 2).

CONCLUSION

The application of high density SNP assays to genotype animals of an ovine resource population showed high utility to provide high resolution mapping information for fine-mapping approaches. The results shown here did verify previous identified highly significant linkage or association on chromosome 25. In future studies we will implement population data and genetic similarity among offspring into the analysis. Results will also be tested using more families of the same sheep population.

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