IN-SILICO APPROACH IDENTIFIED POLYMORPHISM ASSOCIATED WITH WOOL TRAITS IN SHEEP

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SUMMARY

A number of factors contribute to the economic revenue of sheep farmers, but breed improvement via selection is one factor affecting the efficiency of sheep production particularly in relation to wool. The complexity of traits, with partly opposing relationships makes it difficult to select for improved economic values of wool. A further investigation of the genetic background of wool quality, quantity as well as pigmentation traits might assist to unravel the basis of this relationship. Our approach was to identify and analyse possible candidate genes in major linkage regions. Using a combination of positional mapping and literature finding of the gene function, we identified lysosomal trafficking regulator (LYST) as strong candidate gene. Polymorphisms were identified using in-silico screening of scaffolds on the virtual sheep genome assembly v2.0. One of four polymorphisms analysed in the ovine LYST gene was significantly associated with clean fleece weight and coefficient of variation of fleece diameter. However, this polymorphism explained only a small proportion of the phenotypic variation, contradicting unpublished findings of major effects of QTL on chromosome 25 for the same traits. Further analysis is needed to analyse the function of LYST and to find additional genes either having a direct effect on wool quality and quantity or regulating the function of the ovine LYST gene.

INTRODUCTION

Largely as a consequence of the antagonistic relationship between two of the major determinants of profit (fleece weight and fibre diameter), the rate of genetic improvement of sheep bred primarily for wool production has been relatively slow (Purvis and Franklin 2005). A better knowledge on the genetic background of wool quantity and quality traits might assist increasing the gain from the farming of wool-sheep by understanding some of the basis for this opposing relationship. A number of linkage studies have identified major loci for wool quality and quantity traits in sheep. Many QTL have been reported for wool quality parameters such as clean or greasy fleece weight and yield and for wool quality including fibre diameter, staple length, coefficient of variation and standard variation of fibre diameter across many chromosomes (Parsons et al. 1994; Allain et al. 1998; Beh et al. 2001; Ponz et al. 2001; Allain et al. 2006; Bidinost et al. 2006; Bidinost et al. 2008 ). Preliminary studies using a resource population have also verified highly significant QTL for wool quality and quantity on chromosome 25 and a meta-assembly of QTL in sheep has suggested major genes on chromosomes 3 and 25 (Raadsma et al. unpublished).

The study presented here aimed to identify polymorphisms in the main candidate region on chromosome 25 using published sequence information and to analyse the association with wool quality and quantity traits in an ovine sheep resource population.

MATERIALS AND METHODS

Animals. For the analysis presented here, a total 170 wether backcross progeny from a resource population of crosses between Awassi and Merino sheep (Raadsma et al. 2009) were used. A total of five wool quality and three quantity traits were recorded from wool samples collected at 75
weeks of age. Measurement of most fibre characteristics were performed by Riverina Wool Testers in Wagga Wagga, Australia (http://www.wooltesters.com.au/).

Genotyping. In a previous conducted linkage study using backcross Awassi x Merino x Merino (AMM) sheep, QTL were identified for wool traits across different chromosomes using the program QTL-MLE (Raadsma et al. unpublished). Highly significant QTL were identified on chromosome 25, where many QTL were located around 15 cM with a 1-LOD drop off confidence interval of 0-32 cM. The region around microsatellite marker DIK2451, where most of the QTL were identified (Raadsma et al. unpublished) was used to identify the most likely location of an underlying candidate gene. Genes on the virtual sheep genome assembly v2.0 (http://www.livestockgenomics.csiro.au/perl/gbrowse.cgi/vsheep2/) were further taken into consideration if their described function suggested a connection to wool development. Scaffolds from the ovine whole-genome sequence on the virtual sheep genome assembly v2.0 were screened for potential SNPs in the candidate gene. A total of four SNPs identified in the region were genotyped using the iPLEX system (Sequenom).

Association analysis. Association was tested using analysis of variance in R (version 2.15.1) where the SNPs were fitted as fixed effects. A number of traits were investigated including greasy fleece weight, clean fleece weight (CFW), fleece yield, fibre diameter, standard deviation of fibre diameter, coefficient of variation (CV), percentage fibres greater than 30 µm and fleece rot. No additional effects were included in the models as all animals were from the same resource flock and kept under the same conditions. Animals were from the same sire.

RESULTS AND DISCUSSION

Previously a number of QTL were identified for wool quality and quantity in the AMM resource population (Raadsma et al., unpublished). In particular, the many QTL on chromosome 25 were located within the same marker intervals. QTL on this chromosome were previously published in a Sarda x Lacaune backcross population (Allain et al. 2006), Merino sheep (Bidinost et al. 2006; Bidinost et al. 2008), and animals from the synthetic INRA401 breed (Ponz et al. 2001). Genome-wide-association studies using data from the AMM animals validated these results and the regions were further fine-mapped (data not shown). The in-silico analysis of the candidate region on chromosome 25 suggested lysosomal trafficking regulator (LYST) as a potential candidate gene. A total of four SNP were reported on the published sequence including a non-synonymous (G/A) polymorphisms in exon 29, two non-synonymous SNP (T/C and A/G) in exon 36 and one synonymous SNP (G/A) in exon 20. All four SNP segregated in the genotyped animals from the Awassi Merino population.

LYST has been previously been related to melanosome formation, a process positioned between melanocyte development and pigment production in the development of pigmentation cells. Association analysis showed that the non-synonymous SNP in exon 29 of the ovine LYST gene was significantly associated (P < 0.01) with CFW and CV. However, none of the other polymorphisms were significantly associated with the investigated wool traits. Additionally the explained variation of the phenotype was rather small using these models. The unpublished study of QTL for wool traits using animals from the same population suggested a major gene especially for wool quality traits, explaining up to 70% of the phenotypic variation for CV for example (Raadsma et al. unpublished). Despite our findings suggesting some effect of the polymorphisms within the ovine LYST gene, we assume that the significant polymorphism in our study is unlikely the major gene underlying the strong QTL identified earlier (Raadsma et al. unpublished). The in-silico approach taken for this study was surprisingly successful as all four polymorphisms identified in the published ovine sequence segregated in our population. We conclude that it is a useful approach screening such data as useful source for preliminary studies. However, further
screening of other genes or genes regulating the function of LYST might be useful to continue future efforts to identify loci underlying the major QTL for wool quality and quantity traits.

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REFERENCES