HIGH RESOLUTION MAPPING OF QUANTITATIVE TRAIT LOCI ON OVINE CHROMOSOME 3 AND 20 AFFECTING PROTEIN YIELD AND LACTATION PERSISTENCY

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SUMMARY

QTL regions associated with lactation persistency and protein yield in sheep have been identified on OAR3 and OAR20. Genotyping of 8 out of 15 existing microsatellite markers on OAR3, and 5 out of 9 on OAR20, in the QTL region of interest, over additional backcross and double backcross daughters, resulted in high resolution mapping of these two QTL regions to 10 cM and 20cM intervals respectively using Linkage Analysis (LA). An additional 28 informative markers (12 on OAR3 and 16 on OAR20) have been genotyped and combined Linkage Analysis/ Linkage Disequilibrium analysis (LA/LD) will be used to obtain further resolution in these regions so that positional candidate genes can be identified. From standard QTL analyses animals with contrasting genotypes '*Q*-' and 'q-' for desired QTLs have been identified for use in functional (transcriptome) studies to obtain a list of differentially expressed genes. The transect of both positional and functional (differentially expressed) genes should lead to a short list of positional functional candidate genes for further analyses and comparative mapping to dairy cows.

INTRODUCTION

An extreme breed-cross of Awassi and Merino sheep has been used as a ruminant model for quantitative trait loci (QTL) mapping of dairy performance traits that are either difficult to measure in dairy cattle due to design limitations or are not recorded by the Australian Dairy Herd Improvement Scheme (ADHIS). Previous mapping studies have identified several QTL affecting milk and related traits on OAR3 and OAR20 (Barillet et al. 2005). In addition, homologous positions on cattle chromosomes BTA5, BTA11 and BTA23 have also been reported to harbour QTL for protein percentage and milk yield on 'The Bovine QTL Viewer', (http://bovineqtlv2.tamu.edu/home.ph). The online combined QTL map of dairy cattle traits also lists QTL for milk yield and persistency in the corresponding cattle chromosomes (Khatkar et al. 2004). With the development of high density genetic maps for a number of livestock species, high throughput genotyping techniques and the advancement of statistical methodologies, it is now possible to fine map QTL and look for causative genes. This study aims to identify genes affecting complex lactation traits by gene mapping and transcript profiling approaches.

MATERIALS AND METHODS

326 ewes from a primary backcross family of a single F_1 Sire from a resource population based on an extreme breed back-cross and inter-cross design between Awassi fat-tail sheep and Merino superfine and medium wool sheep (Raadsma et al. 1999) had been genotyped using 206 microsatellite markers. Initial analyses reported QTL of interest on OAR3 and OAR20. In order to better define the QTL

location and effect, further genotyping was performed in these 2 QTL regions using eight of the fifteen existing markers OARCP34 (38.6cM), TGLA77 (55.6cM), BM8118 (69.9cM), BMS710 (79.7cM), TGLA67 (85cM), DIK4796 (105.4 cM), BM304 (128.9 cM), BM827 (154.5) on OAR3, and five of the nine existing markers, DIK4865 (30.2cM), BASS17 (41.1cM), DYAB (48.7cM), DIK4895 (58.2cM), BM1815 (70.8cM) on OAR20, on additional backcross and double backcross progeny (n=364).

Marker order and map distances were estimated using the CARTHAGENE software (de Givry et al. 2004). This was followed by the construction of paternally and maternally inherited marker haplotypes for each recorded individual. Lactation curve modelling using the Wood (1967) model and a nonlinear mixed model approach was used over lactation records across multiple lactations to derive the phenotypic traits used in the analyses. Within family QTL analysis was conducted using QTL-MLE (an in-house QTL mapping program written in R) (Thomson et. al. 2007). Results were concluded to be significant when LOD scores generated by QTL-MLE were larger than 2 and ranked p-values were less than 0.05.

RESULTS AND DISCUSSION

Sheep QTL regions associated with lactation performance for protein yield and lactation persistency have been identified and refined on OAR3 and OAR20 after within family QTL analysis using the additional genotyping information of 8 out of 15 and 5 out of 9 microsatellite markers (Figure 1). The locations for significant QTL for protein yield and persistency at day 45 are presented in Table 1.

Table 1. Summary of QTL Results

OAR	Traits	Closest Marker	Position cM (2 LOD Support Interval)	LOD score	QTL effect (σp)
3	Protein (%)	DIK4796	95 (89, 101)	2.14	-0.64
3	Persistency r(45)	DIK4796	95 (90, 101)	2.00	-0.56
20	Protein (%)	DIK4895	51 (42, 62)	2.77	0.55
20	Persistency r(45)	DIK4895	51 (43, 62)	2.32	0.58

With the use of additional progeny the QTL confidence interval has been reduced from about 75 cM to 12 cM on OAR3 and about 50 cM to 20 cM on OAR20. Further mapping resolution will be achieved by adding 28 new microsatellite markers (12 on OAR3 and 16 on OAR20) (Figure 1) on backcross and double backcross population (n=708) of Sire 1 (n=364), Sire 2 (n=161), Sire 3 (n=68) and Sire 4 (n=115) in these regions for ongoing LA/LD analysis.

The probabilities of the F_1 sire transmitting the QTL allele *Q* (A=Awassi-derived) versus *q* (M=Merino-derived) conditional on the actual phenotypic and marker data were investigated to identify extreme animals for total milk yield. The genotype information for markers on OAR3 and OAR20 was identified for animals with *MM* genotype, with probabilities of less than 0.1 and lower than average milk yield for the low probability group, and animals with *AA* genotype with probabilities more than 0.9 and higher than average milk yield for the high probability group. An example of segregation of *A* and *M* alleles, indicating an association with the QTL can be seen for marker INRA132 located close to the QTL peak (Figure 2).
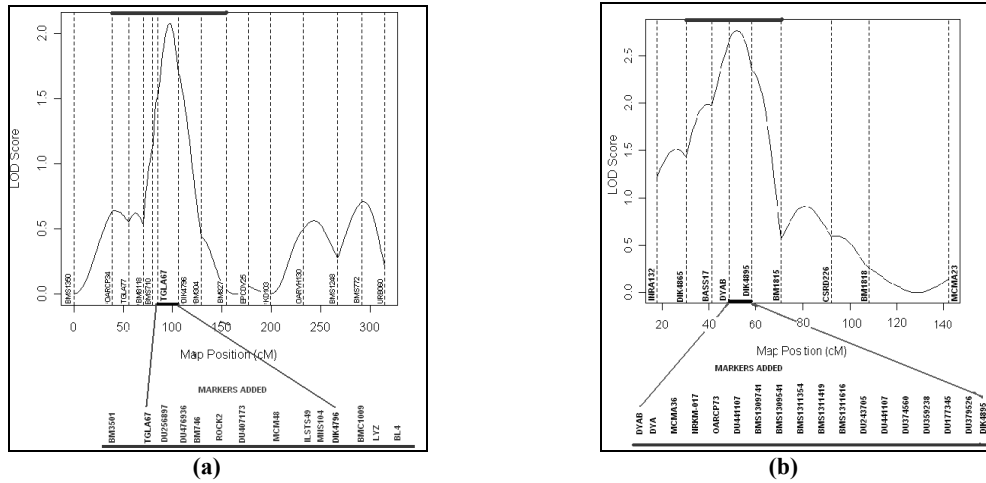
Figure 1. Significant QTL found between markers TGLA67 and DIK4796 (LOD = 2.1, QTL Position = 97 cM) on OAR3 (a) and between markers DYAB and DIK4895 (LOD = 2.8, QTL Position = 51 cM) on OAR 20 (b). Additional markers genotyped in the regions of interest are shown.
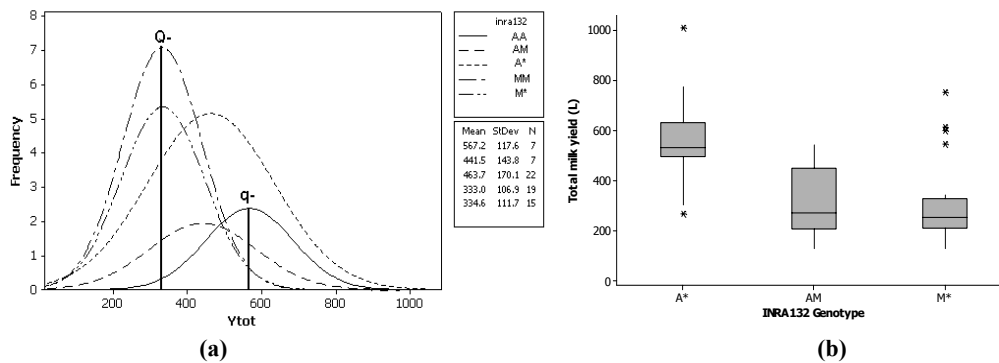

Figure 2. Example of means (a) and distribution (b) of QTL contrast animals defined using genotype information for INRA132 on OAR20.

Eight animals representing extremes for the trait and showing related favourable and unfavourable alleles were obtained using similar information over all the markers based on their genotype and trait information. Tissue samples from mammary glands have been taken from these animals at three different times corresponding to pre-, peak-, and post lactation and snap frozen for gene expression studies.

Figure 3 gives a brief outline of the gene discovery plan for this study. Genes that represent functional candidates will be obtained by means of expression profiling using the bovine Affymetrix microarray Genechip analysis in animals of contrasting QTL genotype (i.e. *Q* vs. *q*). Independent confirmation of selected differentially expressed genes will be achieved by real-time PCR. Functional

and positional evidence for importance of the particular genes on the trait of interest will be identified by integrating positional candidate genes from QTL fine mapping and genes identified against location of functional candidate genes obtained from the transcript profiling (Yagil and Yagil 2006; de Koning et al. 2007). Further analysis of candidate genes and additional SNPs will be carried out using the LA/LD approach. Comparative mapping will then be used to identify similar genes and bovine associated SNPs in a Dairy Commonwealth Research Centre cattle DNA genome resource comprising 2000 samples from all major sire families in the Australian dairy cattle population.
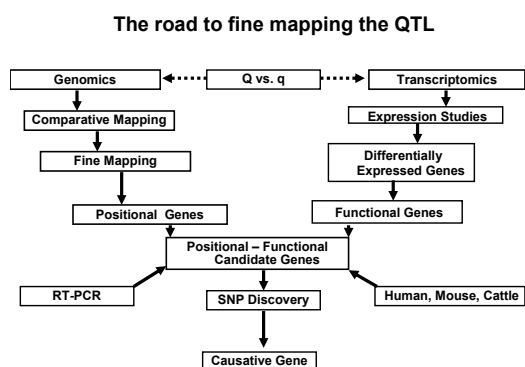
The road to fine mapping the QTL

Figure 3. Flow chart for identification of positional functional candidate genes

CONCLUSIONS
Sheep QTL regions located on OAR3 and OAR20 associated with lactation performance in Dairy sheep will be mapped to a resolution of about 1cM. Ongoing LA/LD analysis will position the QTL to the best possible location within the existing resource. The use of combining QTL mapping and functional analyses using animals of defined QTL genotype will allow a list of positional functional candidate genes for further investigation. Identifying the genes and causative mutation(s) will not only provide the most accurate markers for selection but will also open the possibilities of investigating and manipulating the critical biochemical pathways.

ACKNOWLEDGMENTS

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