COMPARISONS OF QUANTITATIVE TRAIT LOCI (QTL) DETECTED FOR FAT DEPOSITION IN SHEEP USING COMPUTED TOMOGRAPHY

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SUMMARY
New genetic technologies have enabled us to begin the process of unravelling the underlying genetic architecture for complex traits. We report on the identification of quantitative trait loci (QTL) for seven fatness traits at various depots derived by computed tomography (CT) scanning. Progeny (n=164) from a backcross between Merino and Awassi sheep were used in an attempt to exploit the differences that exist in the fat attributes between the two breeds. A genome-wide scan using 117 informative microsatellite markers was used. With the exception of fat depth and %internal fat, all fat traits were found to be highly correlated. Six key QTL areas were detected with one in common to all traits highlighting the polygenic nature of fatness.
Keywords: QTL, sheep, fat, genome-wide scan

INTRODUCTION
Application of advanced knowledge underlying complex production traits may lead to novel breeding technologies such as marker-assisted selection (MAS) where animals may be selected for favourable genes based on closely linked markers or where the gene is known, direct selection for favourable alleles. Identification of genes that have a large effect on traits of economic importance and their function is also a pre-requisite for alternative means of gene manipulation or control. MAS and gene manipulation are likely to be of interest for traits of high economic value and where selection is difficult and/or expensive. Fat deposition plays an important role in sheep meat production and in particular affects efficiency of production. Excessive fatness results in inefficient use of feed resources, and losses by meat processors, along with low consumer acceptance. A number of studies have reported a major gene or gene effect for increased muscling and fatness in Texel (Marcq *et al.*; Marshall 1999; Nicholl 1998), and Dorset sheep (Nicholl 1998). This provides an opportunity to exploit major gene effects to increase the efficiency of selection and production. To date the major QTL reported for body composition in sheep are muscularity traits, while only a few are for fat traits (Walling *et al.* 2002). This is in contrast to QTL detected in other species, where for example in the 2001 Human Obesity Gene Map (Rankinen *et al.* 2002), 165 QTL have been reported for traits associated with obesity, including both mouse and pig QTL.

Fatness QTL in sheep are of interest since fat traits (internal fat, carcass fat, total fat) are difficult to measure and have to be measured later in life. Although there is much information on the distribution of fat as a tissue component (see for example Thompson and Ball (1997), to date there has been very little published on fatness QTL in sheep.

MATERIALS AND METHODS

The ReproGen sheep mapping resource population (Raadsma *et al.*1999) utilizes extreme breed differences between the Merino and the Awassi sheep to map genes for milk, wool and carcass composition. In particular the two breeds differ in regard to growth rate and meat production with the Merino having a smaller frame size combined with major differences in fat deposition and distribution by comparison with the fat tail Awassi.

The Awassi ´ Merino population. Four Awassi sires were crossed with medium and superfine Merino ewes to produce F1 progeny assumed to be heterozygous for target QTL. Four F1 ram lambs were selected to represent each of the founder families and were backcrossed to super fine and medium Merino ewes to produce four families with approximately 400, 150, 150, 150 progeny. This experiment utilized the wethers from the first sire family (n=164).

Genotyping. A genome-wide scan was conducted using a panel (245) of pre-selected polymorphic micro-satellite markers, of which 136 were informative. On completion of genotyping, 117 markers provided complete information across all animals covering the 26 autosomes. The number of markers per autosome varied between two and nine, with an average distance between markers in the range of 20-65 Centimorgans (cM) for each chromosome. The average marker density across the genome was 40cM with a predicted genome coverage greater than 70%.

Phenotyping. *Live animal measures.* All wethers were randomly allocated to two management groups and grown out to two (n=85) and three (n=79) years of age at slaughter. These two groups of sheep were grazed on pasture up to three and six months prior to slaughter, respectively. At this time all sheep were fed *ad libitum* on a grain/lucerne diet as part of a feed intake study.

CT measurements. Prior to slaughter all animals were analysed by Computed Tomography (CT) for measurement of body composition. The animals were restrained and scanned three days prior to slaughter. Cross-sectional images were collected every 40mm starting proximal to the femur/tibia articulation and finishing at the first cervical vertebra. A total of 24 to 28 images were collected, depending upon the length of the sheep. Each image was copied, cropped (to remove viscera and internal fat) and stored separately. From the information of all processed images, the internal fat [Intfat] (comprising kidney, pelvic, mesenteric and heart fat), subcutaneous fat depth [Subfat], carcass fat [Carcfat] (a combination of subcutaneous, inter- and intra-muscular fat depots associated with the carcass), and total fat [Totfat] (comprising all fat depots), were estimated using the PC based CT image analysis program AutoCAT (Jopson *et al.*1995). Subcutaneous fat depth was taken around the eye muscle from to the 1st lumbar vertebra and the next caudal image 40mm apart. The measurement was taken at the point 2/3 the width of the eye muscle, ventral to the midline and the average of the two depths calculated. Percentage total fat [%Totfat] and percentage internal fat [%Intfat] were calculated from the total image-derived weight. However, carcass fat [%Carcfat] was the percentage of fat in the carcass only (i.e. excluding viscera and internal fat). Bodyweight [Finalwt] was also measured at scanning.

Genetic and statistical analyses. *Map distance.* Genetic marker distances were calculated using CRI-MAP (Green *et al.,* 1990). Marker information was compared with the sex-averaged ovine genetic map (Maddox *et al.,* 2001) and found to be consistent in marker order and distance.

QTL analysis. Online QTL analysis was conducted using 'QTL express' (Seaton *et al,* 2002)(using the half-sib servlet). Genotype probabilities for each animal were calculated at 4cM intervals along each chromosome. A chromosome wide threshold for statistical significance was calculated based on a permutation test of 1000 iterations. The analysis included an adjustment for age as a fixed effect.

RESULTS AND DISCUSSION

The two cohorts showed a substantial difference in the amount of fat ($p<0.01$). This is most likely due to the second group being fed for an extended period *ad. Libitum* and additionally they were 12 months older.

Six QTL were identified which had a significant effect on one or more of the fat traits (see Table 1). The QTL effects varied from 0.51 to 0.88 phenotypic standard deviations, with the Merino allele resulting in a consistently lower degree of fatness at all depots. One QTL affected all measures of fatness, implying that a major gene may be responsible for general fatness in the sheep. The other five QTL affected fatness at specific depots.

Table 1. **QTL effects (in units of phenotypic st.dev) and significance levels for seven fat traits**

	Trait						
QTL	Totfat	%Totfat	Carcfat	%Carcfat	Intfat	%Intfat	Subfat
1	ns	ns	ns	ns	0.71 *	ns	ns
2	ns	ns	ns	ns	ns	ns	0.52 *
3	ns	ns	ns	ns	0.69 *	0.88 **	ns
4	0.51 *	0.75 *	0.64 **	0.87 **	0.55 *	ns	0.72 *
5	Ns	0.63 *	0.55 *	0.69 **	ns	ns	ns
6	0.51 *	ns	ns	ns	ns	ns	ns

* $0.01< p <0.05$ ** $p<0.01$ ns - Non significant

Since QTL 4 influenced all fat traits and the other QTL influenced more specific regions a correlation analysis of the traits was conducted to examine the relationship between the various depots. Table 2 shows the phenotypic correlations between fat traits and their relationship to total body weight at time of slaughter. As was expected, carcass fat and total fat were highly correlated with final weight. Of interest in the results is the relatively low correlation between subcutaneous fat depth and the other fat depots. Although positively correlated, this possibly indicates that fat depth is describing a separate trait. Selection on the QTL affecting subcutaneous fat without having a significant influence on the other fat depots (QTL 2 in Table 1) may result in reduced subcutaneous fat with no decrease in total or carcass fat.

There is considerable evidence in the literature indicating differences between sheep breeds in fat partitioning (Donald *et al.* 1970). Frutos *et al* (1997) and Thompson and Ball (1997) suggest that dairy

breeds have a proportionally greater amount of non carcass fat (i.e. internal fat) relative to carcass fat compared with meat breeds. Our results are consistent with this view since the two QTL for increased internal fat are inherited from the Awassi which is a dairy breed.

Table 2. Phenotypic correlations between fat traits adjusted for year effect

	Finalwt	Totalfat	%Totalfat	Intfat	Intfat%	Carcfat	%Carcfat
Totfat	0.856						
%Totfat	0.654	0.905					
Intfat	0.675	0.852	0.807				
%Intfat	0.423	0.662	0.735	0.932			
Carfat	0.863	0.954	0.851	0.729	0.496		
%Carfat	0.653	0.865	0.922	0.669	0.543	0.909	
Subfat	0.491	0.588	0.602	0.435	0.327	0.638	0.656

In humans there is a large amount of evidence suggesting that the genetic determinants of fatness and in particular obesity are extensive. In the 2001 Human Obesity Gene Map Rankinen *et al.* (2002) report on six human and six mouse genetic mutations linked to obesity. In addition 165 QTL associated with obesity related traits are reported. It is highly likely that adiposity in sheep may have similar genetic complexity as is seen in human and mouse studies.

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