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**Genome-wide scan for carcass composition as assessed by X-ray computed tomography (CT) in Scottish Blackface lambs**

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**ABSTRACT:** About 600 Blackface lambs born from 2001 to 2003 had their carcass composition assessed using X-ray computed tomography (CT). GWAS was conducted using the Ovine 50k SNP chip on the CT traits that included muscle, fat and bone weights and their proportions as well as muscle and fat densities. Genetic parameter estimates were obtained using both the pedigree and genomic relationship matrix. We obtained moderate to high (0.19-0.78) heritability estimates. GWAS identified some SNPs reaching the genome-wide significance threshold. Both bone weight and bone area had associated SNPs located on ovine chromosome 6 (OAR6) with p-values of 5.55x10⁻⁸ and 2.63x10⁻⁷, respectively. We also identified a region on OAR1, having an effect on fat. On OAR1, we identified SNPs associated (p=7.28x10⁻⁷) with muscle density. Bone trait SNPs potentially provide an opportunity for fine mapping, and for studying bone growth and malfunction. **Keywords:** Carcass composition; CT traits; GWAS

**Introduction**

The aim of genetic improvement is not only improving the efficiency and profitability of farming enterprise, but also delivering to the consumer a product that is both healthy and gastronomically enjoyable. For lamb to gain or maintain its competitiveness in the meat market place, consumers need assurances in terms of healthiness, composition and taste of the product. Improvements in product quality will include identification of measurable carcass composition and meat quality traits. In addition, methodologies that will translate the resultant knowledge back to sheep breeding programmes need to be identified.

Previous studies have identified that carcass composition traits assessed using X-ray computed tomography (CT) are heritable and that they are correlated to both growth and meat quality traits (Karamichou et al. (2006a), Lambe et al. (2008)). Quantitative trait loci (QTL) for both carcass composition and meat quality have been reported in the literature for studies using microsatellite markers (Karamichou et al. (2006a,b,c), Cavanagh et al. (2010)). However, carcass and meat quality traits are hard to measure, and genetic improvements could be implemented through identification of genes or QTL of interest or alternatively by using genomic selection using abattoir data linked back to the producers. The objective of the study was to provide information that could be used to enable such breeding programmes. We explored genetic architecture underlying meat quality traits as measured by CT using the Ovine 50k SNP chip.

**Materials and Methods**

**Data.** Available data were from 751 pedigree-recorded Scottish Blackface lambs born between 2001 and 2003 from a flock comprising previously selected fat and lean lines, established in 1988 (Bishop (1993)).

**Phenotypic measurements.** *In vivo* carcass composition measurements (600 lambs) were obtained once a year, over 3 days at an average age of 24 weeks using CT. These measurements included muscle, fat and bone weights and their proportions as well as the muscle and fat densities from cross-sectional scans of the ischium (ISC). The same measurements were also taken at the 5th lumbar vertebra (LV5) and the 8th thoracic vertebra (TV8). Live weight (LW) was also recorded at the time of the CT measurements. Using prediction equations from a previous calibration study (Scottish Agricultural College (SAC), unpublished) on unrelated Scottish Blackface lambs of the same age, the following traits were calculated: predicted fat weight, predicted muscle weight, predicted bone weight, predicted total carcass weight, fat proportion, muscle proportion, bone proportion, fat area at the cross sectional scan of the ischium (mm²), muscle area at the cross sectional scan of the ischium (mm²), bone area at the cross sectional scan of the ischium (mm²) and their densities (Karamichou et al. (2006a)).

**Genotypic data.** All animals were genotyped with the 50k SNP chip. After quality control (QC) for minor allele frequency (MAF) less than 0.05 and call rate at 0.90, a total of 40264 SNPs remained for further analyses. SNP positions were obtained from the Sheep Genome browser v3.0 (http://www.livestockgenomics.csiro.au/sheep/).

**Model.** The fixed effects accounted for effects of year (2001-03), sex (male and female), litter size (singles and twins), age of dam (1-4+ years), management group (1,2), line (1-7) and as covariates effects of dates of birth and slaughter. Trait data were checked for normality and where appropriate transformed either using their square root or their natural logarithm.

**Genetic parameter estimates.** The genetic parameter estimates were obtained using ASReml (Gilmour et al. (2009)), fitting the fixed effects given in the model and individual as random effect, using either the complete available pedigree or the genomic relationship matrix.
**Association Study.** GWAS analyses were run using the GenABEL package (Aulchenko et al. (2007)). For each trait, mixed models, comprising fixed effects, the first three principal components (PC) and polygenic effects were fitted using the mmscore function. After Bonferroni correction, the genome-wide (p<0.05) and the suggestive (i.e., one false positive per genome scan) significance thresholds were p<1.24 x 10^{-6} and 2.48 x 10^{-7}, respectively.

**Results and Discussion**

**Genetic parameter estimates.** Moderate to high (0.19-0.79) estimates of heritability using the pedigree were obtained for CT traits. Other CT studies have reported moderate heritability estimates (0.25-0.36) for carcass fat, carcass muscle and bone and moderate to high genetic correlations with weaning weight (Lambe et al. (2008)). However, these authors found low genetic correlation between ultrasound muscle depth and CT traits. In a previous study on the same animals as in the current study, Karamichou et al. (2006a) reported high genetic correlations between CT traits and meat quality. The high correlations, in principle, would enable selection responses for correlated traits in the more expensive to measure traits.

**Association Study.** GWAS analysis identified four traits with SNPs that reached p<0.05 genome-wide threshold (Table 1). The most notable traits being those associated with bone (Figure 1). Both bone area at ISC and bone weight had SNPs located on ovine chromosome 6 (OAR6) with p-values of 2.63 x 10^{-9} and 5.55 x 10^{-8} respectively. Interestingly, there was also a region on OAR6 that seemed to have an effect on fat weight when live weight was fitted as a covariate (Figure 2). Although there was another clear region on OAR6 for fat area at TV8 when accounting for LW, it only crossed the suggestive significance threshold (data not shown). Previously, a partial genome scan analysis using microsatellite markers and linkage analysis, by Karamichou et al. (2006b) using the same data did not include OAR6. However, they identified QTL on bone traits at OAR1 and OAR20. In a more recent study, using the body growth traits measured in the same animals, Riggio et al. (2013) identified several SNPs found in our study on OAR6 to be also associated with body weight at 16, 20 and 24 weeks of age using mixed model association analysis. They also found two regions, significant at the suggestive threshold, within our region of interest, that were associated with body weight at 16 weeks of age using regional heritability mapping approach (RHM). Karamichou et al. (2006a) found intramuscular fat content to have a positive genetic correlation with juiciness and flavor, and a negative genetic correlation with shear force. In another study using microsatellite markers and CT traits, Cavanagh et al. (2010) identified putative QTLs for internal fat, percentage fat and lean in carcass, total fat, carcass fat and final body weight on OAR6. However, in the same study, unlike in our study, they found QTLs for carcass bone, carcass lean and percentage lean in carcass on OAR1. Interestingly, we identified SNPs on OAR1 associated (p=7.28 x 10^{-7}) with muscle density at TV8 region (Figure 3). Previously, Karamichou et al. (2006a) found that muscle density was the CT trait most consistently related to meat quality traits and had moderate to strongly negative genetic correlations with live weight, fat class, subcutaneous fat score, dry matter proportion, juiciness, flavor and overall liking. A live weight QTL on OAR1 was reported by McRae et al. (2005) in Charollais commercial sheep and the same QTL was later confirmed in independent commercial Charollais and Suffolk sheep (Matika et al. (2011)). Several QTLs for lamb flavor, slaughter weight, bone density at ISC, hot carcass weight and meat colour were mapped to OAR1 (Karamichou et al. (2006b)).

![Figure 1: Plot for bone area ISC using GenABEL mmscore (black dashed line represents genome-wide significant threshold and red dashed line the suggestive threshold.)](image)

<table>
<thead>
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<th>Trait/SNP name</th>
<th>Chr</th>
<th>Location</th>
<th>P_value</th>
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<tr>
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<td>s66995</td>
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</table>

Table 1. SNPs associated with CT traits from genome-wide association analysis after correcting for the genomic inflation factor λ.
SNP annotation. We scanned a 1MB region around our highest p-value SNP on OAR6 using the current Ensembl browser (http://www.ensembl.org) that uses sheep genome Ovis_aries_v3.1 and we identified a possible candidate gene OST, an osteopontin precursor, which maps to position 36651734 to 36658288 bp and plays a role in bone formation. However, on OAR1, there was no obvious candidate, except for KAT2B which is implicated in cell growth and differentiation.

Conclusion

CT-assessed carcass traits have moderate to high heritability estimates. In our study we identified two regions on OAR1 and OAR6 that were associated with muscle, fat and bone traits. There is evidence of CT traits being genetically correlated with meat quality traits. Selection using SNPs identified in our study will have implications on both growth and meat quality traits. The high GWAS peak found for bone traits potentially provides an opportunity for fine mapping, and this region could be used as model for studying bone growth and malfunction.

Acknowledgements

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Literature Cited


