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GENOME WIDE ASSOCIATION STUDY USING THE OVINE SNP50 BEADCHIP AND LAMBS SELECTED FOR EXTREMES FOR CARCASS LEAN MEAT YIELD

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SUMMARY

A phenotype resource of extremes for carcass lean meat yield in lambs was established in an attempt to identify regions of the genome associated with carcass lean meat yield. Data were available on 1150 lambs genotyped using the Ovine SNP50 BeadChip. Only two SNPs reached nominal significance (P-values of the order 10^{-8}), with both on Chromosome 2 in the region of GDF8, the gene which contains the already known GDF8 c.1232 *G>A* mutation. The c.1232 *G>A* SNP is not on the Ovine SNP50 BeadChip, with the closest SNPs 10 to 30kbp away, however, these proximal SNPs do not appear to be in LD with c.1232 *G>A* and were not identified in the analysis. The most significant SNPs identified actually lie 2 to 4Mbp away from GDF8, but are in higher LD with c.1232 *G>A*. Models were developed to test whether or not the significance of the SNPs was due to their LD with c.1232 *G>A*. The c.1232 *G>A* genotypes, fitted in models, explained a large proportion of the difference, however, there was still significant residual carcass yield variation explained by these SNPs. This suggests the presence of other SNPs within GDF8 or in neighbouring genes affecting carcass yield, a hypothesis supported by work in cattle and other sheep resources. Using the resource developed no other genomic regions containing significant QTL were identified. The ability to detect other smaller QTL that account for less of the genetic variation may require an increased sample size and/or information from higher density SNP chips.

INTRODUCTION

Data generated from the Ovine SNP50 BeadChip (www.sheephapmap.org) can be used in several ways, including the development of Molecular Breeding Values, or the identification of genomic regions which explain either all of the variation in monogenic traits or a large amount of variation in polygenic traits. For the former, there are already a number of publications that report the identification of the gene causing various monogenic disorders such as Arthrogryposis, Achondroplasia and Progressive Muscular Dystrophy through the selective genotyping of case and control animals using the Ovine SNP50 BeadChip. Such studies often require only small numbers of animals exhibiting the disorder to be genotyped. There are fewer published reports on the ability of the Ovine SNP50 BeadChip to detect regions that explain variation in polygenic traits; one such report is the identification of SNPs on OAR4 associated with average daily gain, staple length, wool grade, and fleece weight in Rambouillet sheep (Hadfield *et al.* 2012). The genotyping of different breeds using the Ovine SNP50 BeadChip has also been used to look at breed differentiation and signature sweeps, and one often reported is the signature of Texel sheep in the region of Myostatin (GDF8) on OAR2 (Kijas *et al.* 2012).

An industry-derived data set was collected for animals containing phenotypic extremes for meat yield with the aim of identifying genomic regions that have a large effect on the trait.

MATERIALS AND METHODS

A description of the data set has previously been provided by Johnson *et al.* (2011). Briefly, data were collected in 2008 and 2009 at Alliance Group Ltd meat plants. Lambs were selected from large mobs of greater than 200 lambs, with carcass weights between 15.5 and 19kg. One to three of the most extreme yielding pairs of animals (high and low, matched for carcass weight) were identified from a total of 344 mobs. No information about breed, age or origin was available

on the lambs. Measurements recorded on the whole carcass are described by Johnson *et al.* (2011) and included carcass weight, carcass length, buttock circumference (BC) and VIAscan® carcass measurements of the lean meat yield of the leg, loin, and shoulder expressed as a percentage of the carcass weight, together with their sum total.

The lambs were genotyped using the Illumina OvineSNP50 BeadChip (www.sheephapmap.org), and for the GDF8 c.1232 *G>A* variant with data analyses were performed using the R package GenABEL (Aulchenko *et al.*, 2007). Illumina OvineSNP50 BeadChip genotype and phenotype data were available on 1,150 lambs made up of pairs of high and low yielding extremes representing 344 mobs. The data for each trait was adjusted for sex, year of birth, and mob together with the first six principle components fitted to the autosomal SNPs from each animal's 50K SNP data. A polygenic model using a kinship matrix was calculated, by the 'ibs()' function of GenABEL using the weight = 'freq' option, from all autosomal makers as identity by state (Aulchenko *et al.* 2007).

The residual from this mixed model was tested for association with each SNP independently (Chen and Abecasis, 2007). Principal component analysis (PCA; prcomp function in R) or classical multidimensional scaling (CMD) was used to calculate principal components or co-ordinates to check for further population structure and outliers.

Further investigation of SNPs identified as significant was conducted using SAS (SAS, 2004), using the General Linear Model procedure based on models described by Johnson *et al.* (2011), with the SNP of interest fitted as a fixed effect. An additional model for each SNP involved firstly fitting the GDF8 c.1232 *G>A* genotype of the animal as a fixed effect.

RESULTS AND DISCUSSION

Of the traits assessed the most significant results were observed for BC with evidence of a large peak on Chromosome 2 (Figure 1). Two SNPs (s02728 and OAR2_128772350) contributing to this peak had nominal P-values of the order 10^{-8}, a level of significance of interest in GWAS studies (Barsh *et al.* 2012). There were also peaks on Chromosome 2 for other lean meat yield related traits. For example, total yield estimated by VIASCAN® had similar results to BC, with the most significant SNPs, which were generally the same SNPs as for BC, having nominal P-values in the order of 10^{-7}. Interestingly, no associations of this order of significance were discovered outside of the region of the GDF8 locus for the traits analysed.

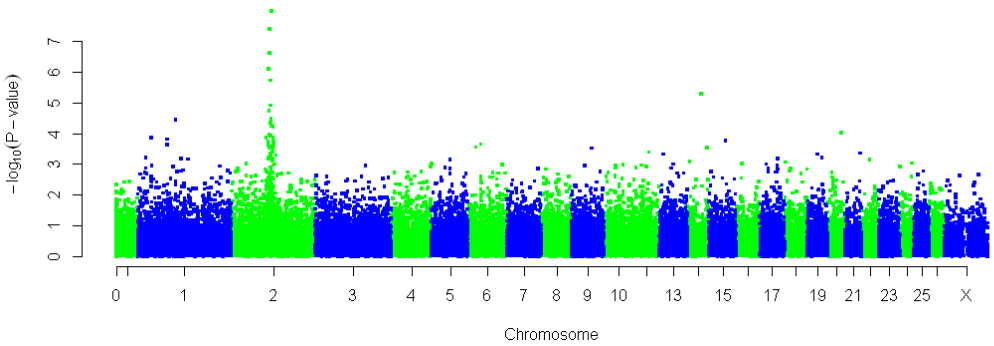

Figure 1. Manhattan Plot for Buttock Circumference in a Lean Meat Yield Extremes Industry Derived Sheep Resource

The region defined by the most significant peaks contains GDF8 (Figure 2, Top), with the SNPs 4Mbp and 2Mbp either side of GDF8. The known GDF8 c.1232 *G>A* mutation derived from

Texel sheep are not on the Ovine SNP50 BeadChip, with the closest SNPs (OAR2_125305996 and OAR2_126354465) 10 and 30kbp either side of GDF8 (Figure 2, Bottom). The effect of c.1232 *G>A* on carcass traits in this resource was reported by Johnson *et al.* (2011). Whether or not the SNPs identified are simply acting as markers for c.1232 *G>A* was investigated through looking at the level of LD between the SNPs (results not presented). This analysis showed that there was actually a higher level of LD (but not perfect) between the significant SNPs identified in the analysis and c.1232 *G*>A than the most proximal SNPs and c.1232 *G>A.*

The results from further analysis of the most significant SNPs are in Table 1 and Table 2 where the SNPs are fitted separately, and then with c.1232 *G>A* genotype fitted in the model prior to the inclusion of the SNP. Table 1 shows that most of the variation explained by the SNPs can be attributed to their LD (even though not perfect) with c.1232 *G>A*, but that there is also evidence of other mutations within this region affecting lean meat yield, via the presence of a residual significant effect. The presence of other mutations related to lean meat yield in the region is supported by evidence from cattle for multiple mutations within GDF8 and evidence from Johnson *et al.* (2005) for a second QTL in the region of GDF8 in Texel sheep.

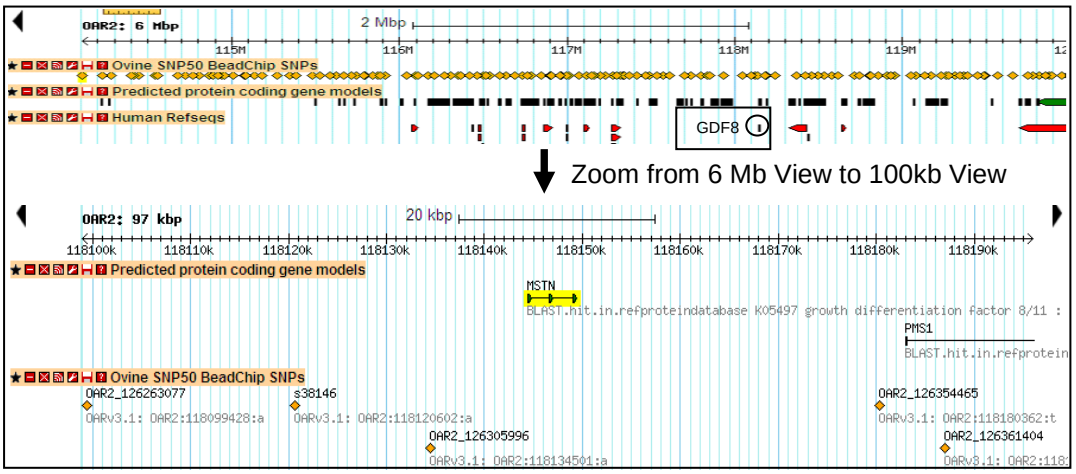

Figure 2. Illustration of Ovine Chromosome 2: Top, region that is bounded by the two most significant SNPs (s02728 and OAR2_128772350); Bottom, an enhanced view of the region immediately around GDF8 (MSTN)

Table 1: Significance of associations between Buttock Circumference (cm) and SNPs identified using the OvineSNP50 BeadChip, before and after inclusion of GDF8 c.1232 *G>A* genotype in the model

SNP	GDF8 c.1232 G>A Fitted First in Model	Partial R2	P Value	LSMeans For Genotype[1]		
				1	2	3
GDF8 c.1232 G>A		0.11	P<0.0001	64.4	63.4	62.4
s02728		0.11	P<0.0001	64.3	63.3	62.3
OAR2_128772350		0.13	P<0.0001	64.4	63.5	62.3
s02728	✓	0.02	P<0.0001	63.9	63.5	62.8
OAR2_128772350	✓	0.03	P<0.0001	64.0	63.6	62.7

[1] 1 and 3 represent the two homozygous genotypes, and 2 represents the heterozygous genotype

Table 2: Significance of associations between Total ViaSCAN® (lean?%) and significant SNPs identified using the OvineSNP50 BeadChip before and after inclusion of GDF8 c.1232 *G>A* genotypes in the model

SNP	GDF8 c.1232 G>A Fitted First in Model	Partial R2	P Value	LSMeans For Genotype[1]		
				1	2	3
GDF8 c.1232 G>A		0.14	P<0.0001	58.0	55.3	52.9
s02728		0.14	P<0.0001	57.9	55.1	52.9
OAR2_128772350		0.14	P<0.0001	57.7	55.4	52.8
s02728	✓	0.02	P<0.0001	56.9	55.4	54.2
OAR2_128772350	✓	0.02	P<0.0001	56.4	55.9	54.3

[1] 1 and 3 represent the two homozygous genotypes, and 2 represents the heterozygous genotype

CONCLUSIONS

Genotyping of carcass lean meat yield extreme lambs using the Ovine SNP50 BeadChip confirmed the region of GDF8 Ovine Chromosome 2 as influencing lean meat yield. In addition this study provides evidence for other mutations in the region that affect carcass lean meat yield, in addition to GDF8 c.1232 *G>A*, albeit they have a smaller effect.

That no other genomic regions were identified suggests there are unlikely to be QTL of a similar magnitude, at a reasonable allele frequency, segregating in the population studied. Increasing the sample size and/or the use of information from higher density SNP chips may be valuable to examine other regions in the genome that have QTL that account for less of the genetic variance in the traits studied.

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