A case-control study to identify a genetic component contributing to wet carcass syndrome in sheep

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Summary

Wet carcass syndrome (WCS) is a condition predominantly found in lambs which negatively affects the quality of carcasses. During the pre-slaughter period, the animal appears to be clinically normal, showing no symptoms of an abnormality. However, after slaughter and removal of the skin the carcass appears to be “wet”. The condition is described phenotypically as a subcutaneous accumulation of watery fluid. Therefore, the objective of this investigation was to scan the genomes of afflicted and unafflicted lambs in search of putative quantitative trait loci associated with the WCS phenotype in sheep. Muscle samples from lamb carcasses (43 afflicted and 41 unafflicted) were collected from three different slaughterhouses in the Northern Cape Province of South Africa and Southern Namibia. To test for an association between the phenotype (WCS) and an autosomal genetic marker, a case-control study design was implemented. Separate analyses for each sex were motivated by individual SNP on the X chromosome being suggestive of a QTL. These analyses revealed significant associations between SNP and WCS in males, but not in females. The three SNPs reaching genome-wide significance in males are in strong linkage disequilibrium (LD) with the HTR2C and TENM1 genes.

Keywords: association analysis, carcass, single nucleotide polymorphisms

Introduction

Wet carcass syndrome (WCS) is a condition predominantly found in lambs which negatively affects the quality of their carcasses. It is most frequently observed in Dorper and crosses of Dorper with indigenous and locally developed breeds of South Africa and Namibia (Brock et al., 1983; Webb & van Niekerk, 2011). During the pre-slaughter period, the animal appears to be clinically normal, showing no symptoms of an abnormality. However, after slaughter and removal of the skin the carcass appears to be “wet” (Hattingh et al., 1983). The condition is described phenotypically as a subcutaneous accumulation of watery fluid (Brock et al., 1983). Afflicted carcasses pose difficulties during meat processing and the meat has a reduced shelf life (Joubert et al., 1985). Therefore, these carcasses are deemed unacceptable and are condemned by the meat inspectors. In South Africa, economic losses stemming from WCS were approximately 27 million Rand in 2010 (Webb & van Niekerk, 2011; le Roux, 2012).

While WCS has been recognized since 1981 and the subject of research since that time (Brock et al., 1983; Hattingh et al., 1983), its ethology remains undetermined. Not all animals within a flock sent to slaughter at one time are afflicted and inducing the condition
experimentally has so far proved infeasible. To date, many physiological and nutritional factors, as well as environmental agents and management systems have been evaluated without success. However, despite variation in the incidence of WCS among breeds being potentially indicative of genetic causation, there are no prior investigations of a potential genetic predisposition to WCS. Therefore, the objective of this investigation was to scan the genomes of afflicted and unafflicted lambs in search of putative quantitative trait loci associated with the WCS phenotype in sheep.

**Material and methods**

**Data and quality control**

Muscle samples from lamb carcasses of the Dorper breed (43 afflicted and 41 unafflicted) were randomly collected from three different slaughterhouses slaughterhouses in the Northern Cape Province of South Africa and Southern Namibia. Afflicted- and unafflicted samples were collected from the same cohort, however sex of the animals was not recorded. Samples were genotyped using the Ovine Infinium® HD SNP BeadChip (Illumina, San Diego, California, USA). The chip contains 685 734 SNPs that are uniformly distributed across the ovine genome. Genotyping was performed at the Agricultural Research Council - Biotechnology Platform (ARC-BTP), Pretoria, South Africa. The software package, PLINK v1.07 (Purcell et al., 2007) was used for subsequent analyses.

Individual animals were removed from the dataset if the call rate for an individual SNP was < 0.90. In addition, identity by descent (IBD) was calculated for all possible pairs of animals and one member of the pair was removed from the dataset if IBD was > 0.1875. Markers without a known chromosomal position on the ovine genome sequence (Ovis aries Oar_v4.0) were excluded. Additional markers with call rates < 0.90 and minor allele frequency (MAF) < 0.01 were also excluded. Sex was assigned to animals based on the presence (F ≤ 0.2 = female) or absence (F ≥ 0.8 = male) of heterozygosity of the X chromosome. Following this editing, 71 samples (40 males and 31 females) representing 35 afflicted and 36 unafflicted animals, and 552 490 SNPs remained for further analyses.

**Association analysis**

To test for an association between the phenotype (WCS) and an autosomal genetic marker, a case-control study design was implemented using the Fisher’s Exact tests within PLINK v1.07 (Purcell et al., 2007). To adjust for multiple testing, the standard Bonferroni correction (Abdi, 2007) was applied in which the adjusted p-value was obtained by dividing the observed p-value (0.05) by the number of loci tested (n = 552 695) which resulted in a p-value of 9.05 x 10⁻⁸ as a threshold for significance. Following this initial analysis, similar analyses were conducted for the males (21 afflicted and 19 unafflicted) and females (14 afflicted and 17 unafflicted) separately. The ‘qqman’ package (Turner, 2014) within R (https://cran.r-project.org) was used in the creation of the Manhattan plots.

**Results and Discussion**

The analysis of all data revealed a small number of suggestive associations. However, there was no convincing evidence for a quantitative trait locus. A principal component analysis (revealed) no population sub-structure within the sampled groups (results not
presented). Separate analyses for each sex were motivated by individual SNP on the X chromosome being suggestive of a QTL (\(p = 2.874e-007\)). These analyses revealed significant associations between SNP and WCS in males (Table 1, Fig. 1), but not in females (Fig. 2). Given the possibility of X-inactivation (Clayton, 2009) the data arising from females was edited to remove heterozygous individuals at each locus. This further reduced the number of observations and coupled with relative low minor allele frequencies precluded testing the independence of homozygous genetic classes and WCS in the females.

Almost all SNPs were situated within genes. The three SNPs reaching genome-wide significance (i.e. \(p < 9.05 \times 10^{-6}\)) are in strong linkage disequilibrium (LD) with the \textit{HTR2C} and \textit{TEMNI} genes. Both of these genes play a role in the plasma membrane of cells and influence biological processes such as cellular calcium ion homeostasis, behavioural fear responses, and signal transduction (https://www.ensembl.org/Ovis_aries/). Given the physiological description of the WCS phenotype i.e. the subcutaneous accumulation of watery fluid, and the role these genes play in the plasma membrane by likely influencing cell homeostasis, suggests that these genes are positional and functional candidates for susceptibility to WCS.

\textit{Table 1. Significant single nucleotide polymorphisms, their chromosomal position, and p-value for their association with wet carcass syndrome in males.}

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\textit{List of References}


Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Sham, P.