Genetic architecture of resistance to virulent ovine-footrot in a case-control study of New Zealand Merino sheep

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Abstract

The genetic architecture of resistance and susceptibility to virulent ovine footrot was studied in a cohort of 3,208 animals from 37 informative flocks of predominant Merino (93%), and Merino Types (Poll Merino, Dohne and South African Meat Merino). Footrot was scored as a binary trait where an animal was scored as affected (1) or unaffected (0, free from footrot), after 2 known challenges. For final analysis, animals from flocks with a prevalence in the range of 30-70% were selected. Animals were genotyped with either a 50K or 15K SNP panel on the Illumina Ovine bead array resulting in combined imputed SNP genotype for 51,713 markers for all animals. The animals were of mixed ages (lambs, yearlings, hoggets and adults), sexes, and breeds. Heritability of footrot was 0.39 ± 0.04 based on a genomic relationship matrix on the underlying scale. Corresponding proportional chromosome heritabilities were in the range of 0.00 ± 0.01 (OAR12) to 0.14 ± 0.04 (OAR23) and compared against expected based on chromosome length. Two markers on OAR23 accounted for a significant component of the additive genetic variance, whilst all residual SNP markers failed to reach statistical significance, despite being located on chromosomes with disproportionate effect on the genetic variance. The polygenic nature of genetic variation in resistance to footrot is discussed.

Keywords: Footrot, GWAS, Merino, SNP, genetic architecture, heritability

Introduction

Ovine footrot is an infectious and, on specific occasions, an exceptionally contagious disease resulting from invasion of epidermal tissue of the hooves by a mixed group of bacteria. An essential component of this mixture is *Dichelobacter nodosus*. The disease is characterised by infection of the interdigital skin (IDS), and may under certain conditions progress to separation of the sole, soft and hard horn from the underlying hoof matrix. In severe cases footrot is a debilitating disease associated with acute lameness. It impacts on animal welfare, and economic costs associated with lost production and control, therapeutic and preventative measures. The expression of footrot in a flock of sheep is governed by three factors: a) virulence of *D. nodosus,* b) the suitability of the environment for predisposition of the host and transmission of the organism, c) inherent susceptibility of the host. These factors have been described in detail by Egerton and Raadsma (1991). Genetic variation in resistance to footrot has most recently been reviewed by Raadsma and Connington (2011) and within the Merino breed the heritability of resistance is within the range of 0.2-0.3. Current developments in genomic technologies now allows us to dissect this genetic variation to study the underlying genetic architecture contributing to resistance and susceptibility to

footrot. This paper reports on one such study in a commercial population of NZ Merino sheep where footrot is endemic and of economic impact.

Materials and Methods

Data source

Footrot data was collected from 4,543 animals from 62 flocks across the south island of New Zealand. The protocol aimed to collect 40 affected and 40 unaffected animals per farm, with a maximum allowance of 80 infected and 80 clean per farm. The footrot phenotype was binary, where an animal was scored as clean (0) when all four feet scored 0 after two known challenges, or affected (1) when at least one foot was shown to exhibit severe footrot (score 4+, Raadsma and Connington, 2011). No further discrimination between scores in the severity of infection were made. The final analysis was completed on 3,208 animals from 37 informative flocks which had to a flock prevalence of footrot in the range of 30-70% and complete genotype information. The animal's ages (lambs (3%), yearlings (6-12months) (15%), hoggets (12-18months)(16%) and adults (65%)), sexes (16% male), and breeds (Merino (93%), poll Merino, Dohne and South African Meat Merino) varied across but not within flocks. Animals were genotyped with a mixture of the 50K marker SNP panel on the Illumina Ovine bead array (~75%) and the Ovine 15K Illumina panel (~25%). After removal of SNP for QC checks (Call rate <85%, MAF<0.05), a combined imputed SNP genotype for 51,713 markers was constructed for all animals using F-impute.

Genomic relationship matrix (GRM) and variance component analyses.

No traditional pedigree was available for this data set and all analyses were performed using the genomic relationship matrix (GRM) according to VanRaden (2008). Parameter estimations were conducted using ASReml software (Gilmour *et al.*, 2009). Estimates for the binary trait were obtained on the observed scale using a linear model and on the underlying scale a logit link function. Flock, sex, breed and age class were fitted as fixed effects. A single random genetic effect was fitted for each animal using the GRM. The genetic variance accounted for by each chromosome and the corresponding heritability, was calculated using a GRM derived from all the SNP assigned to a specific individual chromosome in turn and the GRM from all residual SNP. Expected heritability (proportion of total genetic variation) was calculated for each chromosome assuming that gene effects were all equal and evenly distributed along the genome, and chromosome effects were proportional to chromosome length. For each chromosome, observed chromosomal heritability was plotted against expected heritability.

GWAS and false discovery rate.

For each animal the complete bi-allelic genotype was fitted as a fixed effect after fitting breed, flock, age class and sex as fixed effects in WOMBAT (Meyer, 2007) using the SNP snappy function (Meyer *et al.*, 2012) on the observed scale. A GRM based on the genomic information was also fitted as a random term. The derived probabilities were plotted as a Manhattan plot against genome position of each SNP. All derived probabilities were also plotted against expected probabilities using R "qqman" and the False Discovery threshold calculated using "qvalue" in R.

Results

Variance component analysis.

The heritability of footrot on the observed scale based using a full genome derived GRM was 0.39 ± 0.04 with an additive variance of 0.097 ± 0.011 and phenotypic variance of 0.248 ± 0.010. Corresponding estimates on the underlying scale were 0.39 ± 0.04 (heritability), 0.632 ± 0.108 (additive genetic variance), and 1.632 ± 0.107 (phenotypic variance). Partitioning the genetic variance to each chromosome resulted in a range of chromosomal heritabilites from 0.00 ± 0.01 to 0.06 ± 0.01 on the observed scale to 0.00 ± 0.01 to 0.14 ± 0.04 on the underlying scale. In both analyses OAR23 expressed a disproportionately higher heritability than expected, based on chromosome length, with estimates of 0.06 ± 0.01 and 0.14 ±0.04 on the observed and underlying scales respectively (Figure 1) followed by OAR9, 15, and 21(Figure 1).

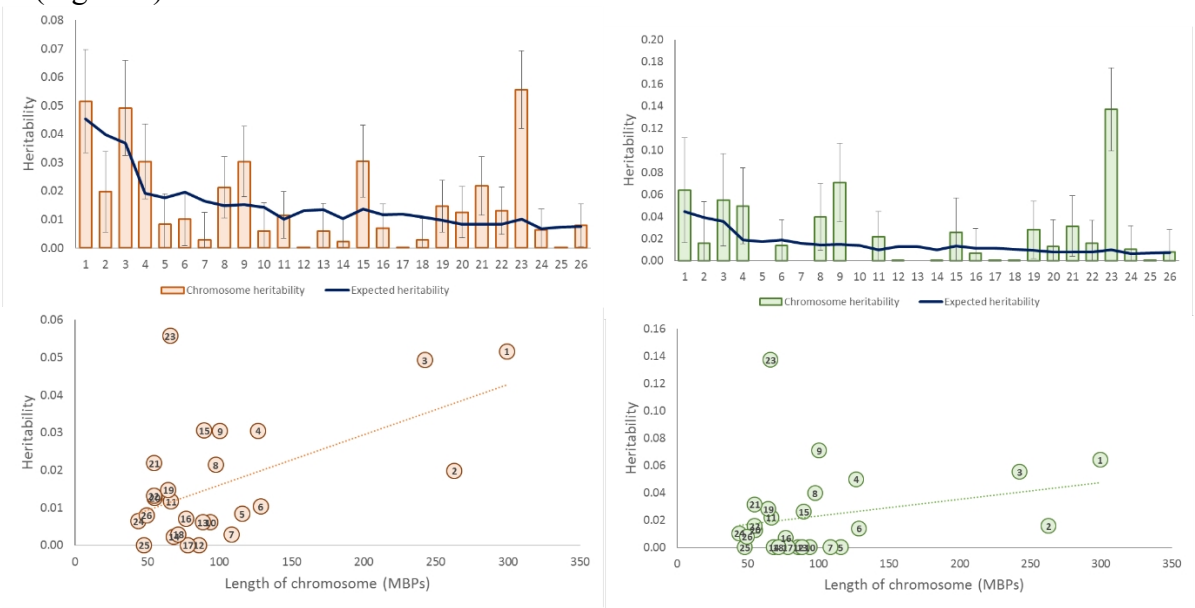

Figure 1: Heritability of chromosomes for footrot on the observed (left - orange) and the underlying (right – green) scale.

The GWAS analyses resulted in 2 SNP on OAR23 (Figure 2), OAR23_27112379.1 (P = 2.66 e^{-13}) and OAR23_27233801_X.1 (P = 6.02 e^{-11}) at position 25,972,928 and 26,109,761 OARver3.1 respectively, being significantly associated with footrot in this data set. Both SNP targeted an adjoining position on OAR23 on the Illumina array. Results for all other SNP associations, including closely flanking SNP on OAR23, fell below the significance thresholds (Figure 2), despite being located to chromosomes which accounted for a disproportionate amount of the additive genetic variance. Fitting the two significant SNPs as fixed effects resulted in a decline in the heritability of footrot on the observed scale from 0.39 ± 0.04 to 0.36 ± 0.04. This coincided with the chromosomal heritability of OAR23 declining from 0.06 ± 0.01 to 0.01 ± 0.01 on the observed scale with the 2 SNP accounting for most of the additive genetic variance. The QTL location corresponded to a gene family DSG2, DSG3, DSG4 and DSG1.

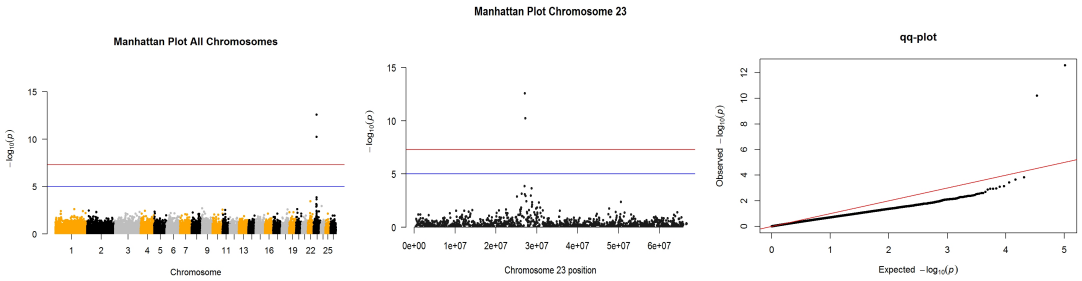

Fig 2 a-c Genome wide association between SNP genotype and footrot in a case control study (a) genome wide probability profile,(b) OAR 23 probability profile, and (c) qq plot for expected and observed SNP association probability.

Discussion

This study provides insights into the nature of genetic variation in host resistance /susceptibility to challenge with virulent ovine footrot. The heritability estimates reported here are higher than those previously reported for Merino sheep, or indeed other breeds (as reviewed by Raadsma and Connington 2011). In particular the similarity between heritability estimates on the observed and underlying scale, may in part be attributed to the fact that animals from both ends of the spectrum of resistance/susceptibility were chosen in flocks with an optimal expression of 30-70% of footrot. Furthermore a balanced set of affected and unaffected animals were selected following exposure to 2 challenges. The heritability estimate may thus have been biased upwards from having both extreme phenotypes and repeat expression of the trait. The flock differences in prevalence of footrot were confounded with season, geographical location, breed, age and sexes of animals under observation and therefore removed from the genetic analyses as a fixed effect.

For the first time we report the partitioning of the additive genetic variance according to chromosomes. We show that not all chromosomes contribute equally to the additive genetic variance, according to chromosome length, which is suggestive that underlying causative genes are not equally distributed across the genome. Chromosomes which accounted for a disproportionate amount of the genetic variation include OAR9, 15, 21 and 23, suggesting more genes related to footrot resistance or genes with large effect located on these chromosomes. Of note is the absence of OAR20 to account for a significant proportion of the genetic variation in resistance to footrot, which is known to contain the MHC and is in contrast to earlier reports by Litchfield *et al.* (1991) and Esayg *et al.* (1993). Although the SNP array may not have covered the full haplotype diversity in the MHC region, SNP density would have been sufficiently high to discriminate between common haplotypes.

We also report that despite several chromosomes being important in resistance to footrot, only one QTL was significantly large enough to be deemed informative in resistance. The location of two relatively closely co-located SNP on OAR23 showed a significant effect on resistance to footrot, and combined they accounted for most of the genetic variation attributable to that chromosome. Overall, the effect of the two SNP was relatively small, accounting for less than 10% of the heritability of resistance to footrot. The detection of a QTL with a significant effect is in contrast to the GWAS reported by Mucha *et al.* (2015). The latter study was also across a wide range of flocks in the Texel breed, albeit of a smaller scale (n=400). It is possible that the QTL reported here may not be segregating in Texel sheep, or their study was underpowered to detect the allelic effect. The nature of the QTL as contributing to either resistance or susceptibility could not be determined from this study as resistance was recorded as a binary trait. The four candidate genes DSCG2, DSCG3, DSG4 and DSG1 were identified immediately under the QTL region of which DSG3 was the lead

candidate, and follow up studies including flanking genes B4GALT6, TTR, DSC1, DSC2, DSC3 and the use of high density SNP genotype arrays is needed, as well as using a broader expression of footrot resistance using the graded scoring system for severity and information from all four feet.

Conclusions

We conclude that significant genetic variation exists in resistance to virulent footrot in NZ Merino and Merino type sheep and that the trait is amenable to selection. We also conclude that genetic variation is polygenetic in nature with potentially many genes of relatively small effect distributed unevenly across the genome. Although a locus on OAR23 may harbour alleles which account for a dis-proportionate amount of the genetic variation in either resistance or susceptibility to footrot, their effect is unlikely to be strong enough as a stand-alone predictor of genetic resistance and a full account for the polygenic nature through use of EBV for resistance is likely the most useful way for industry to capture this genetic variation (Raadsma and Connington 2011; Walkom *et al.*, 2018 these proceedings).

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