Genome-wide association for facial eczema tolerance in New Zealand sheep

K.M. McRae[1], S.M. Clarke[1], T.J. Johnson[1], H.J. Baird[1], S-A.N. Newman[1], K.G. Dodds[1], J.C. McEwan[1] & S.J. Rowe[1]

[1] AgResearch Invermay, Private Bag 50034, Mosgiel 9053, New Zealand
kathryn.mcrae@agresearch.co.nz (Corresponding Author)

Summary

Facial eczema (FE) is a metabolic disease of great importance to the New Zealand sheep industry. Liver and bile duct damage, caused by ingestion of the mycotoxin sporidesmin, results in photosensitisation, and reduced production. There is genetic variation in tolerance to facial eczema, and breeding programs have been successful in making genetic progress. The objective of this study was to utilise large phenotypic datasets in conjunction with low density genotypes, to interrogate the sheep genome for regions associated with variability in tolerance to facial eczema. A QTL on chromosome 15 at the β-globin locus is reported which explains 8% of the genetic variance. Haemoglobin haplotypes have previously been associated with variation in a number of health related traits in sheep.

Keywords: sheep, disease, facial eczema

Introduction

Pithomycotoxicosis, or 'facial eczema' (FE), is a debilitating, often deadly, metabolic disease of ruminants caused by ingestion of the mycotoxin sporidesmin, produced by the spores of the fungus *Pithomyces chartarum*. Ingestion of sporidesmin by livestock, results in liver inflammation and bile duct blockage (Smith & Towers 2002), leading to photosensitization in exposed areas of the animal, particularly in lightly pigmented areas such as the face (Clare 1944). Losses arise from a decrease in production (Smith 2000) and reproduction (McMillan *et al.* 1988; Moore *et al.* 1983; Morris *et al.* 1991) in both clinically and sub-clinically affected animals.

Long-term climate change projections indicate that there will be an increase in the geographical spread of *P. chartarum* in New Zealand, resulting in outbreaks of FE in currently unaffected regions (Dennis *et al.* 2014). Currently, strategies for preventing the severity of FE include protecting animals through ingestion of zinc, spraying pasture with fungicide, pasture management, and breeding animals for tolerance (di Menna *et al.* 2009). Serum gamma glutamyltransferase (GGT) collected 2 to 3 weeks after sporidesmin challenge can be used as a measure of liver damage in the live animal (Towers & Stratton 1978). The heritability of log-transformed serum GGT levels at 21 days after a measured sporidesmin challenge (GGT21) calculated using selection lines was 0.45 (Morris *et al.* 1995), with a small negative genetic correlation with yearling fleece weight (Morris *et al.* 1999). Recent re-estimation of genetic parameters for facial eczema tolerance using a larger dataset found a comparable heritability (0.44), although no correlation between GGT21 and any production traits, including fleece weights (McRae *et al.* 2016). A commercial testing programme, Ramguard, was developed in the mid-1980's to provide New Zealand sheep breeders with sporidesmin and an ethical dosing strategy for ascertaining FE tolerance (Amyes & Hawkes 2014). The resulting GGT21 values are used to generate breeding value estimates for tested

and related animals, with 800 to 1,100 young rams tested each year.

Selection of animals with increased resistance to disease can be expensive and time consuming, and has potential implications for animal welfare. Genomic selection can be used as a complementary approach to current methods for disease control (Goddard 2012), and is currently implemented in the New Zealand sheep industry for a number of traits (Auvray *et al.* 2014), including GGT21 (Phua *et al.* 2014b). Genomics, through tools such as genome-wide association studies, can also be used to further increase our understanding of the genetic mechanisms underlying the host response to disease, and compare these mechanisms between breeds or species. A number of approaches have been used to search for causative genes or loci underlying FE tolerance, including a candidate gene approach (Duncan 2007; Phua *et al.* 1999), genome-wide scans for quantitative trait loci (QTL) (Phua & Dodds 2011; Phua *et al.* 2009) and scanning for selective sweep signatures (Phua *et al.* 2014a). There was only a small amount of concordance between the results of the studies, with two selective sweep regions, on chromosomes one and 13, falling within suggestive QTL regions.

With advancement in technology, the cost of genotyping has reduced significantly, and as a result the number of animals with both genotypes and FE trait measurements (GGT21) in the New Zealand sheep industry has increased. The objective of our study was to use this resource to discover genomic variants associated with FE in New Zealand sheep.

Material and methods

Animals

All animals were selected based on GGT21 records and genotype availability, and were a subset of the dataset used to re-estimate genetic parameters for FE tolerance described previously (McRae *et al.* 2016). All animals had been tested through the RamGuard programme. All data were obtained from Sheep Improvement Limited (SIL), including GGT levels 21 days after a measured sporidesmin challenge (GGT21). The final dataset consisted of 1,981 animals from 39 flocks, born between 2010 and 2014.

Genotyping

Animals were genotyped with the Illumina OvineLD BeadChip (15,000 markers) according to the manufacturer's protocol. Genome coordinates for all SNP were based on the OARv3.1 ovine genome assembly. Quality control checks excluded markers that appeared non-autosomal (including pseudo-autosomal), had a call rate below 95%, and/or had a minor allele frequency (MAF) $\leq$0.05. Individuals were excluded from the analysis if there was more than 5% genotyping failure. After quality control measures, 1,931 animals with LD genotypes were available, with 13,893 out of 15,000 SNPs utilised for analysis.

Genome-wide association study

Genome-wide association analyses (GWAA) were performed using SNP & Variation Suite v8.4.0 (Golden Helix, Inc., Bozeman, MT, www.goldenhelix.com). To investigate SNP markers associated with facial eczema tolerance, GWAA were performed using 1) Efficient Mixed-Model Association eXpedited (EMMAX) analysis using identity-by-state (IBS) and 2) a genotype association test. EMMAX analysis was performed on raw phenotypic data (GGT21), with contemporary group (flock.year.sex.GGT21mob). The genotype association

test was performed on residuals obtained from ASReml (v4.1) (Gilmour *et al.* 2015) after fitting pedigree and the fixed effect of contemporary group. For both analyses, the first two principal components were also fitted as additional covariates to ensure population stratification was taken into account After Bonferonni correction, the threshold for genome-wide significance ($P < 0.05$) was nominal $p < 3.3x10^{-6}$.

To calculate the proportion of variance explained, SNP effects were estimated in ASReml, by fitting SNPs as fixed effects in the model described above. The proportion of V_A due to each SNP was estimated as $(2pq\ (a+d(q-p))^2)/V_A$, where AA, BB and AB are the predicted trait values for each genotype class, estimated using the predict function, p and q are the allelic frequencies at the SNP locus, a is the genotypic value of the best homozygote, d is the deviation due to dominance and V_A is the total additive genetic variance of the trait obtained when no SNP fixed effects are included in the model.

Results and discussion

Low density genotyping of animals with FE phenotypes revealed a QTL on chromosome 15, using both EMMAX and association analyses (Figure 1A and B). The region on chromosome 15 contains two known protein coding genes (*OR51V1* and *OR52Z1*), five unknown protein coding genes, and one small nuclear RNA (Figure 2B). This region has previously been identified as the location of the β -globin locus (Jiang *et al.* 2015).

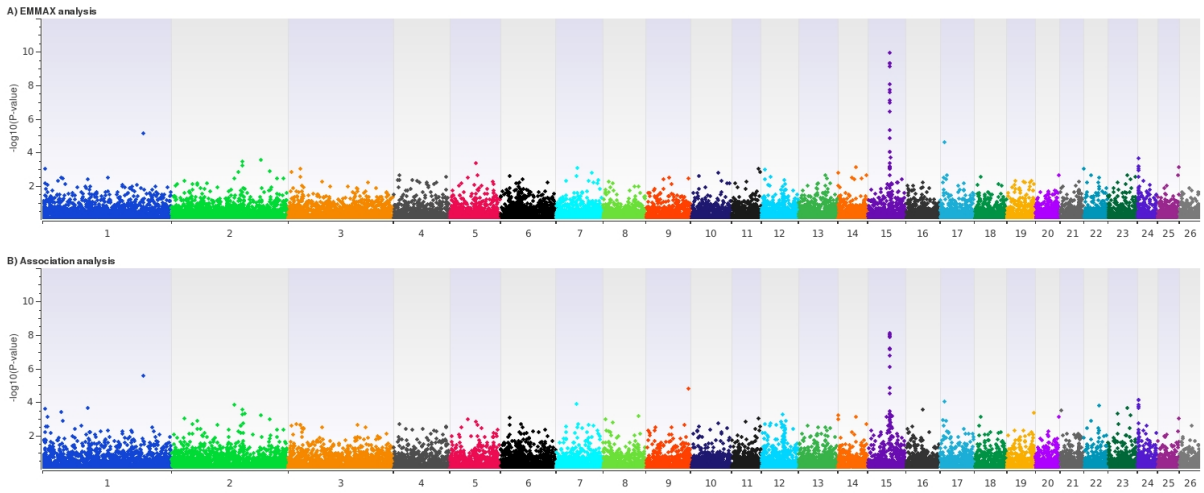

Figure 1. Manhattan plot of genome-wide association analysis for Facial Eczema in New Zealand sheep. (A) Efficient Mixed-Model Association eXpedited (EMMAX) analysis using identity-by-state (IBS) were conducted using raw phenotypic data (GGT21) with contemporary group and the first two principal components fitted as a covariates. (B) Genotype association tests were performed using residuals obtained from ASReml after fitting pedigree and the fixed effect of contemporary group.

The top SNP, rs430842113 (chr 15:47570178), has a minor allele frequency of 0.47 (Figure 2A and C). Initial estimates of the variance explained by the SNP using the genotyped animals in this study were 8% of the additive genetic variance. The SNP is located in the 5' untranslated region of the uncharacterised protein-coding gene ENSOARG00000019174 (Figure 2B). This gene shares orthology with human hemoglobin subunit genes.

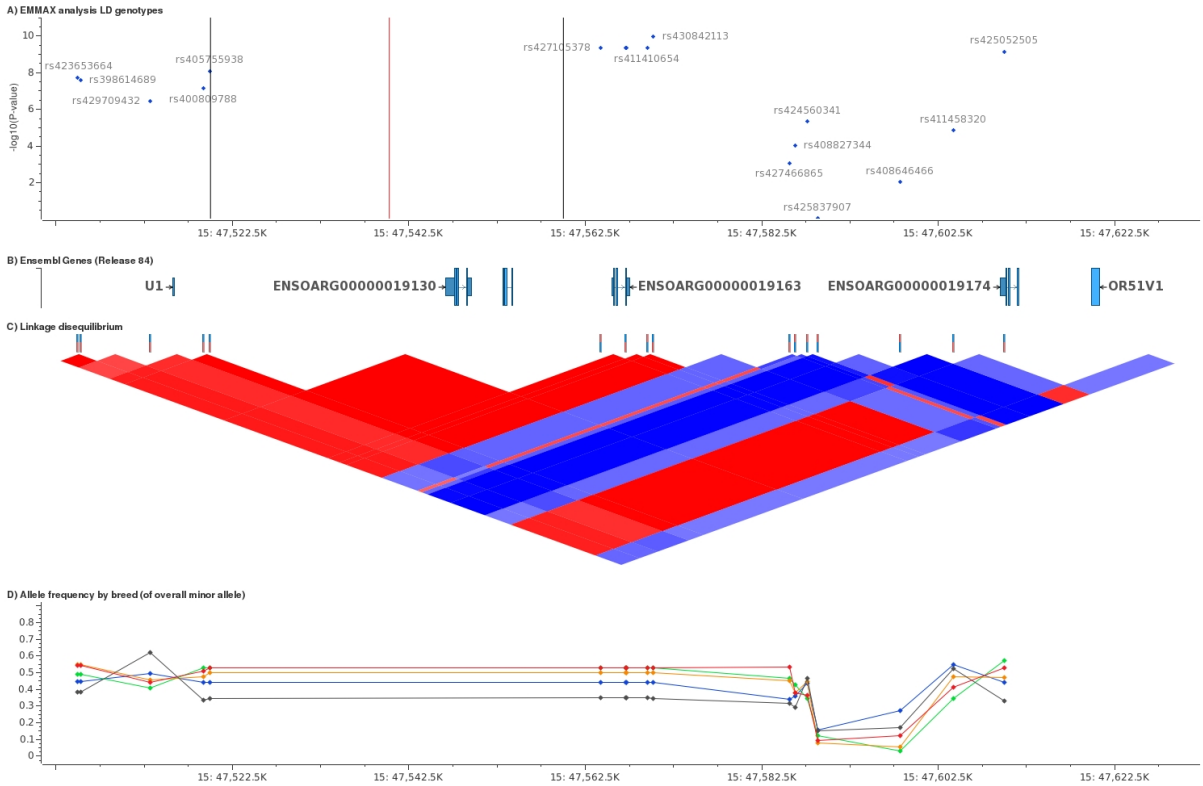

Figure 2. Detailed examination of GWAA peak on chromosome 15 (47,490 Kb – 47,650 Kb). (A) EMMAX analysis using low density (LD) genotypes. The ~37-kb gap in the v3.1 sheep genome assembly previously reported by Jiang et al. (2015) is indicated by the red line. The ~40-kb region of low sequence identity between haplotype A and B (reference genome) is shown by the two black lines. (B) Known genes based on Ensembl Release 84. (C) Linkage disequilibrium, from 1 (red) to 0 (blue). (D) Allele frequency by breed, based on overall minor allele. Coopworth, blue; Highlander, green; Perendale, orange; Composite, grey; Romney, red.

In sheep, the β-globin locus has two different haplotypes, A and B (Evans *et al.* 1956). The blood of sheep homozygous for haplotype A possesses a higher affinity for molecular oxygen than that of haplotype B animals, resulting in a reduced rate of oxygen liberation to tissue (Dawson & Evans 1962; Huisman *et al.* 1958; Meschia *et al.* 1961). The A haplotype has previously been associated with beneficial effects for health related traits including mastitis and parasite resistance in crossbred ewes (Dally *et al.* 1980), and resistance to photosensitisation as a result of ingestion of *Nathercium ossifragum* by Norwegian lambs (Laksesvela & Dishington 1983). Version 3.1 of the sheep genome contains the shorter haplotype B, therefore there are no SNPs on the LD SNP chip used in this study that can be used to determine the haplotypes of the animals in this study. The markers on both sides of the haplotype are in strong linkage disequilibrium, however, therefore they may be predictive (Figure 2C). Previous work looking at the distribution of haemoglobin haplotypes in New Zealand sheep has found both haplotypes present in both Romney and Finnish Landrace breeds (Millar 1980), suggesting that there may be a mixture of A and B haplotypes within the animals in this study.

The results of the additive GWAA show that, even with a relatively low number of animals, this low density ovine chip has the power and utility to detect genomic regions associated with trait variation, prior to imputation to HD genotypes. Further, a novel QTL is

reported on chromosome 15 at the β-globin locus, which explains 8% of the additive genetic variance. Haemoglobin haplotypes have previously been associated with variation in a number of health related traits in sheep, and therefore warrant more investigation into their role in tolerance to facial eczema in sheep.

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