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DIFFERENT METHODS DETECTED DIFFERENT LOCI INVOLVED IN RESISTANCE TO FACIAL ECZEMA DISEASE OF SHEEP

S.H. Phua and K.G. Dodds

AgResearch Invermay, Private Bag 50034, Mosgiel 9053, New Zealand

SUMMARY

Facial eczema (FE) is a secondary photosensitization disease caused by the fungal toxin sporidesmin. The disease affects mainly sheep and cattle, and costs the New Zealand sheep industry alone an estimated $60M a year. In an attempt to develop diagnostic DNA tests for selection of FE tolerant sheep, we have taken different approaches to identify the genes and loci that affect FE sensitivity. These approaches included the candidate gene method, quantitative trait loci (QTL) experiments, analysis of allele-frequencies differences between selection lines (using the Peddrift method of Dodds and McEwan 1997), and a genomic selection (GS) study with ovine 50K-SNP (single nucleotide polymorphism) chips. We detected the involvement of two candidate genes, two QTL regions, three significant SNPs in the Peddrift test and one significant SNP from the GS study. Intriguingly none of these chromosomalsites and regions overlap.

INTRODUCTION

Resistance to facial eczemadisease (FE) is a complex trait. Figure 1 shows a simple conceptual model to depict some of the events that couldbe involved: the depicted biological processes include non-assimilation of sporidesmin from gut, toxin detoxification pathways in liver and the cellular removal of reactive oxygen species. We measure FE trait in terms of the levels of liver-specific enzyme, gamma-glutamyl transferase (GGT), in the blood; under sporidesmin challenge this enzyme is released into the blood stream when cells die, and the blood GGT level is therefore proportional to the extent of liver damage (Towers and Stratton 1978). Hence a GGT measurement reflects the overall outcome encompassing all the processes involved in FE sensitivity. This report summarizes the different approacheswe have taken to identify these FE genes and loci. It should be mentioned that in terms of resistance, there may be other genes and pathways involved in rapid recovery of liver from xenobiotic insult (Phua *et al*. 2009).

MATERIALS AND METHODS

Animals. The background of the Romney FE selection lines was described in Phua *et al*. (1999). Briefly, the lines were established in 1975, and the response of selection was assessed from changes in logGGT (natural log of GGT) breeding value. A total 132 resistant (n=66) and susceptible (n=66) animals were sampled, with birth years range from 1991 to 1995, and the lines'differences were x3.7 (1991) and x6.9 (1995). These animals were used in the Peddrift analysisof candidate gene markers and the Illumina OvineSNP50 BeadChip markers.

In the first QTL experiment (designated RxS), four F1 rams were obtained from reciprocal crosses of resistant (R) and susceptible (S) selection-line animals(Phua *et al*. 2009). These RxS rams were used to generate four half-sib families (with 124 - 168 progeny per family) by out-crossing to unselected Romney ewes. All the progeny were artificially challenged with a fixed dose rate of sporidesmin (0.13 mg/kg live-weight), and their FE trait was measured in terms of blood GGT levels. About 240 microsatellite markers, evenly-spaced throughout the 26 sheep autosomes, were analysed in this study.

In the second QTL experiment (designatedFxT), three rams were generated from crosses of Finnish Landrace (F) rams to Texel (T) ewes. These FxT rams were out-crossed to Coopworth

ewes to generate three half-sib families, having 200 progeny per sire. The progeny were artificially dosed with sporidesmin (0.3 mg/kg live-weight) and their FE trait measured in terms of blood GGTvalues. About 220 evenly-spaced genome-wide microsatellite markers were analysed in this QTL study.

In the genomic selection (GS) study, about 1450 Romney sheep,with recorded GGT trait phenotype, were genotyped across the Illumina OvineSNP50 BeadChip. These were mainly commercial animals collected over thelastten years.

Statistical analyses. In the divergent FE genetic lines, genes conferring sporidesmin tolerance will be selected for in the resistant line and/or against in the susceptible line. As a consequence, the allele frequencies of the genes or markers in linkage disequilibrium with the genes will differ between the two lines. The simulation Peddrift methodof Dodds and McEwan (1997) was used to calculate the significance of contingency table (allele by line) X^2 statistics, by using the actual pedigrees to account for genetic drift due tofounder effects and inbreeding within line. In the Illumina OvineSNP50 BeadChip experiment, after quality control procedures, 50,975 of the SNPs from the chip were analysed.

The QTL method described in Phua *et al*. (2009) was used to analyse the two RxS and FxT experiments. Briefly, genotype data were analysed against logGGT measurements using the interval mapping method of Knott *et al*. (1996); the F-statistic profiles for the regression of phenotype on the conditional probability of inheriting the sires' alleles, were calculated at 2-cM intervals using informative flanking marker genotypes. Genome-wide significant and suggestive thresholds were calculated by permutation (Churchill and Doerge 1994), with at least 1000 replicates.

For the GS study, quality control and analysis methods followed those used by Auvray *et al.* (2011). In brief, logGGT was analysed with gBLUP (animal model BLUP using relatedness calculated from 47,644 polymorphic autosomal SNPs; VanRaden 2008) with a model that included the fixed effects of contemporary group and the first six principal components of the genotypes. The latter were used to account for population structure effects, such as breed differences. Although this resource was aimed at GS, we have used the results to extract preliminary information about individual SNP effects. These effects were obtained from the gBLUP analysis (VanRaden 2008) and their significance determined by assuming these effects are normally distributed with variance proportional to p(1-p) where p is the allele frequency. A Bonferroni adjustment for multiple testing was used ($P<10^{-6}$ was used for genomewise 5% significance).

RESULTS AND DISCUSSION

Aproposed mechanism of sporidesmin toxicity is through the generation of reactive oxygen species (Munday 1989). In the candidate gene approach, we tested some antioxidant genes using the Peddrift method in the FE resistant and susceptible lines and detected the involvement of the catalase gene (Phua *et al*. 1999). Further, an increased expression of pleiotropic drug resistance protein 5 (*PDR5*) was found to confer sporidesmin resistance in yeast *Saccharomyces cerevisiae* (Bissinger and Kuchler 1994); we similarly tested the closest mammalian ortholog of *PDR5*, the*ABCG2*gene (ATP-binding cassette sub-family G member 2 protein, Sheps *et al*. 2004) and found it to be involved in FE sensitivity(Duncan*et al*. 2007) (Table 1).

Two QTL experiments were conducted to identify chromosomal regions carrying FE loci of detectable effect size. The first RxS Romney experiment detected a QTL on OAR3 (Phua *et al*. 2009). The second FxT experiment, involving FE-tolerant Finnish Landrace (F) breed and FE-susceptible Texel (T) breed, identified a QTL on OAR2 (Table 1).

When the ovine 50K-SNP chips became available, we genotyped 66 resistant and 66 susceptible selection-line animals across the chips. Peddrift analysis identified three SNPs, on three different chromosomes, that showed significant allele frequency differences between the lines (P<0.000001) (Table 1).

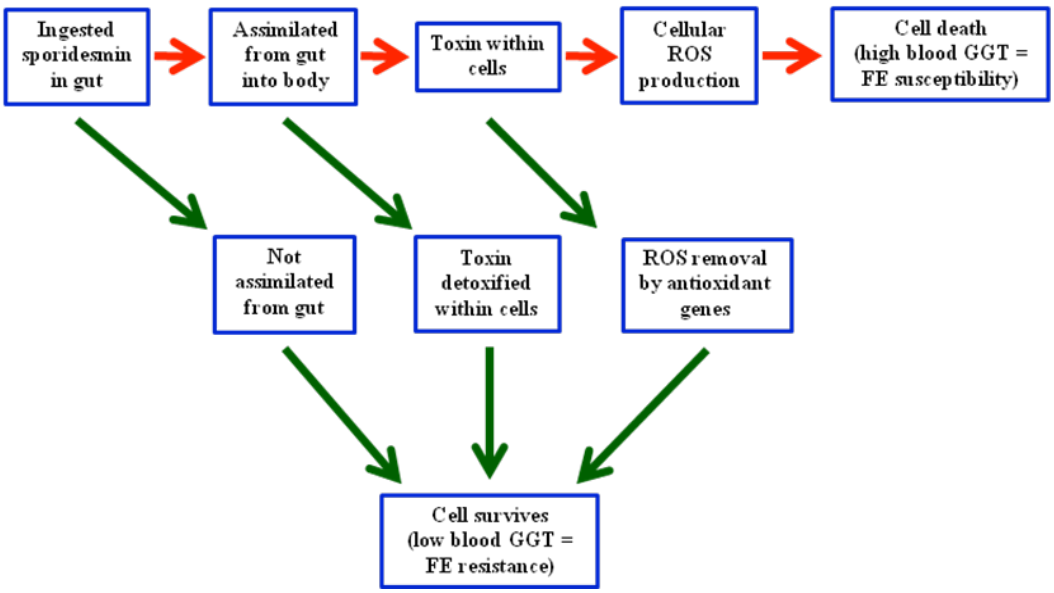

Figure 1. A simplisticmodel showing some of the many processes that could contribute toFE resistance. ROS is reactive oxygen species, and GGT is liver-specific enzyme gamma-glutamyl transferase.

Table 1. Summary of results obtained from different approaches taken to identify FE-causative genes and loci. RxS and FxT are, respectively, QTL studies using FE resistant (R) crossed susceptible (S) rams and Finnish Landrace (F) crossed Texel (T) rams.

Method	Animal Resource	Locus	Autosome	Data
Candidate genes	Romney selection lines	*Catalase*	OAR15	Phua *et al*. 1999
		ABCG2	OAR6	Duncan *et al*. 2007
RxS QTL experiment	RxS Romney families	QTL	OAR3	Phua *et al*. 2009
FxT QTL experiment	FxT outcross families	QTL	OAR2	unpublished
Peddrift test (50K-SNP chips)	Romney selection lines	SNP	OAR1	unpublished
		SNP	OAR11	unpublished
		SNP	OAR12	unpublished
Romney genomic selection study	Commercial Romney animals	SNP	OAR17	unpublished

In the candidate gene approach, Peddrift analyses of markers from the catalase and *ABCG2*genes in the selection lines implicated their involvement in FE sensitivity. But these gene loci, on OAR15 and OAR6 respectively, do not coincide with the OAR3 QTL identified in RxS experiment. An inference is that catalase and *ABCG2* are genes with relatively small effect size. Intriguingly the OAR3 QTL was not in one of the three Peddrift significant SNP regions identified from the 50K-SNP chip experiment. Since the QTL was detected in the half-sib progeny of RxS rams, it is possible that the QTL only functions in the genetic background of the dams. If this is true, it would imply gene-gene interactions. Further, the significant OAR17 SNP site identified in GS study of commercial Romney sheep is completely different from all the regions derived from experimental Romney animals. It appears that different sheep populations may carry different genes affecting their resistance or susceptibility responses to sporidesmin challenge.

In the FxT QTL experiment, we were essentially looking for FE-tolerant genes from Finnish Landrace breed and the susceptible genes from Texel. In view of the Romney results above, it is not surprising to find that the FxT QTL identified on OAR2 does not coincide with any of the FE loci detected in Romney breed.

CONCLUSIONS

The overall results to date suggest that there are at least eight loci contributing to FE sensitivity in sheep. These loci have varying effect size. Because of many biochemical pathways and possible gene-gene interactions, the net effect of an FE locus may depend on the host genetic background. It appears that different sheep populations, particularly different breeds, may carry different FE gene variants.

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