Locating SNPs In The Bovine Genome Associated With Variation In The Immune Response

R.J. Leach[*], S.C. Craigmile[*], O.C. Jann[*], E.J. Glass[*]

Introduction

Infectious disease in livestock is a cause for great concern for both governments and farmers. Although many countries maintain good animal husbandry, farm management and vaccinate their livestock, failure in one or more of these areas allows infectious disease to prevail (Davies G., Genini, S., Bishop, S.C. *et al*. (2009)). For example, Foot-and-Mouth Disease Virus (FMDV) has sporadically caused disease around the EU (Grubman, M.J. and Baxt, B. (2004)), despite bio-security measures. More effective vaccines and the ability to breed for resistance have the potential to provide solutions.

Identifying the genes controlling complex traits such as vaccine induced protection or disease resistance is not straight forward, even though it is clear that variation in these traits has a significant genetic component as shown by ourselves (Glass, E.J. (2004)) and others (Davies G., Genini, S., Bishop, S.C. *et al*. (2009)). Our aims are to identify regions of the genome that explain a significant proportion of observed genetic variance in some of the immune-related traits that underpin vaccine and pathogen responsiveness, and to understand the biological relevance the underlying genes may play during an immune response across time. Our study may lead to the identification of selectable markers associated with increased disease resistance, and also may suggest new ways to design vaccines that enhance the host immune response to pathogens.

The antigen used to elicit an immune response in this study was a 40-mer peptide derived from the VP1 capsid protein of FMDV (FMDV15) which is highly immunogenic and induces protection in a proportion of animals (DiMarchi, R., Brooke, G., , Gale, C., *et al*. (1986)). Protection against FMD is generally believed to relate to the levels of neutralising antibody, but has been correlated with IgG1 and IgG2 levels (Capozzo A, Florio T, Di Loreto S, *et al*. (1997)) and T cell responses (Grubman, M.J. and Baxt, B. (2004)). There is considerable variation in response to this peptide, some of which may be accounted for by Major Histocompatibility Complex (MHC) polymorphisms (Baxter, R., Craigmile, S.C., Haley, C.S. *et al*. (2009)), but it seems likely that other factors are also important. We measured FMDV15 peptide specific IgG1, IgG2 and T cell response during a primary and secondary response following the immunisation of 195 second generation Charolais Holstein backcross heifers. Initially, we conducted a broad Quantitative Trait Loci (QTL) scan using a linkage approach with 165 microsatellite markers across the bovine genome. With the addition of a further 157 Single Nucleotide Polymorphisms (SNP), we fine mapped regions highlighted as significant in the linkage study using a Genome Wide Association Study (GWAS) approach.

[*]The Roslin Institute & R(D)SVS, University of Edinburgh, Midlothian, EH25 9PS, UK.

Material and methods

Animals
501 cross bred second generation animals were produced in the Roslin Bovine Genome (RoBoGen) herd. Immune phenotypes were measured on 121 F2, 43 Holstein backcross (HB1) and 31 Charolais backcross (CB1) heifers. Only female calves were used. The age of initial immunisation with the FMDV15 peptide ranged from 469-609 days.

Immunisation and sampling
The heifers were immunised subcutaneously with 1mg FMDV15 peptide/animal emulsified in Freund's incomplete adjuvant at week 0, and administered a booster of 100µg FMDV15 peptide/animal at week 6. Whole blood samples were collected from all of the heifers at weeks 0, 1, 2, 4, 8 and 10, post immunisation for IgG analysis and at weeks 0, 4, 8 and 10, post immunisation for T-cell measurements.

Phenotypic data
The FMDV15 peptide specific ELISAs were performed to measure IgG1 and IgG2 isotypes as detailed by Baxter, R., Craigmile, S.C., Haley, C.S. *et al*. (2009). The T cell proliferation assay was carried out essentially as described by Glass, E. J., Oliver, R.A., Collen, T., *et al*. (1990) with the exception that whole blood was used.

Statistical Analysis
Stimulation Indexes (SI) were calculated for the T-cell proliferation to the FMDV15 peptide (A_Y) where $SI = A_Y / B_x$ and B_x was the negative control. The IgG1 and IgG2 concentrations and the T cell SI for FMDV15 peptide were normalised to obtain a normal distribution and constant variance (log_{10}). Area under the curve (AUC) for these three traits was also calculated to provide a single trait that reflected the overall response of the normalised data.

REML (REsidual Maximum Likelihood) was used, to determine which factors were significant within the herd. All significant factors were kept in the QTL models for both the linkage and the GWAS.
Thus the final REML model was:

$$Y_{inf} = \mu + \beta + C + \gamma_{minf} + W_{ainf} + u_n + g_f + e_{inf}$$

Where: Y_{inf} is the observed value of the phenotypic trait; μ, population mean; β, the fixed effect of line (3 lines); C, the fixed effect of cohort (3 cohorts); γ_m, the linear covariate of age at vaccination m (m = d469-d609); W_a, the linear covariate of weight a (a= 361-744Kg); u_n, the random effect of sire; g_f, the random effect of dam g; e_{inf}, the residual error.

As the GWAS did not account for the relationships between animals the genetic relationship matrix was broken down into its principle components and principle component 1 was included as a fixed effect in the REML model of the GWAS.

Genetic markers
Each animal in the RoBoGen herd was originally genotyped with 165 microsatellite markers, across all 29 autosomes. A PERL script was written to place all the SNPs for the GWAS

under the peaks of the linkage study, preferentially in clusters and in genes of known immune relevance. A proportion of SNPs were also placed within the QTL regions highlighted by a comparative genomics study as likely candidates for immune-related traits (Jann, O.C., King, A., Corrales, N.L., *et al.* (2009)).

QTL analysis
GridQTL (Seaton, G., Haley, C. S., Knott, S. A., *et al*. (2006)) was used for the linkage study. The analysis was run using the F2 and backcross module with 1000 permutations to obtain significance thresholds. R was used to conduct the GWAS, using the REML model mentioned above. A Bonferroni correction method was used to obtain significance thresholds for the GWAS.

Results and discussion

Table 1: Genome wide significant SNPs and their corresponding trait and SNP number.

T cell proliferation to FMDV15	Chromosome Number	SNP name	Level of Significance
Week 8	6	A	1%
Week 8	6	B	5%
Week 8	6	C	5%
AUC	6	A	5%

The trait, chromosome and SNP location and the SNPs level of significance using genome wide significance.

A total of 63 QTL were located for all 3 of the trait types (IgG1, IgG2 and T cell responses to the FMDV15 peptide) in the linkage study. Of these, 2 were above the genome wide 5% threshold and 1 was above the 1% genome wide threshold (located on chromosomes: 6, 24 and 20, respectively). The GWAS detected 34 significant SNP associations across the bovine genome, the most significant of which were associated with T cell proliferation on BTA 6 (Table 1 and Fig 1), and they have reduced the confidence interval of the QTL in the linkage study. Similar findings were also true for IgG responses with significant SNPs located on BTA 20 (results not shown). Although no significant SNPs were located on chromosome 24 under the peaks of the QTL from the linkage study, the GWAs did refine further significant regions detected in the linkage study, notably on BTA 23 which contains the MHC region, confirming our earlier study (Baxter, R., Craigmile, S.C., Haley, C.S. *et al*. (2009)).

The linkage analysis and the GWAS detected several QTL which influenced the same traits across time, suggesting that there may be gene(s) that impact on both the primary and secondary responses. An example is shown in Fig 1: SNP-A is significant at weeks 8, 10 as well as the overall response (AUC) whereas SNP-B and SNP-C (in linkage disequilibrium) are only significant at week 8.

Conclusion
The linkage analysis together with a GWAS using specifically located SNPs has highlighted regions of the genome as potentially relevant to immune-related traits. The SNPs found in this study may be relevant as markers for disease resistance and the underlying genes may provide new information to improve the efficacy of vaccination.

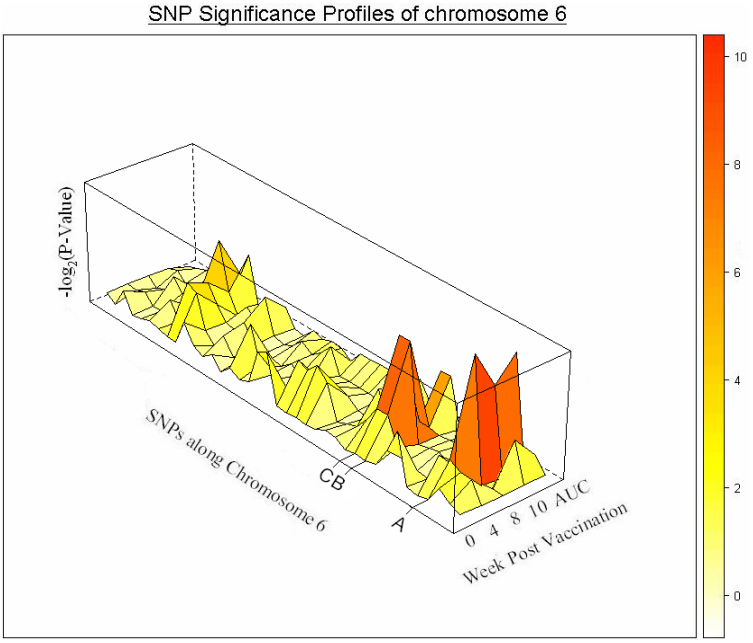

Figure 1: A 3D plot showing the significance (Y-axis) of SNPs located on chromosome 6 (Z-axis) during the time course of T cell proliferation to the FMDV15 peptide (X-axis). The colour key to the right of the plot displays the corresponding magnitude of the plotted P-values ($-\log_2$). Three SNPs, A, B and C are significant at week 8 post vaccination, whilst SNP-A is also significant at week 10 and AUC.

References

Baxter, R., Craigmile, S.C., Haley, C.S., *et al*. (2009). *Vaccine*, 28(28): 28-37.

Capozzo A, Florio T, Di Loreto S., *et al*. (1997) *Vaccine*, 15(6-7): 624-30.

Davies, G., Genini, S., Bishop, S.C., *et al*. (2009). *Animal*, 03: 415-436.

DiMarchi, R., Brooke, G., Gale, C., *et al*. (1986). *Science*, 232(4750): 639-41.

Glass, E. J., Oliver, R.A., Collen, T., *et al*. (1990). *Animal Genetics*, 21(1): 15-28.

Glass, E. J. (2004). *Anim Health Res Rev*, 5(2):197-208

Grubman, M. J. and Baxt, B. (2004). *Clinical Microbiology Reviews*, 17(2): 465-93.

Jann, O.C., King, A., Corrales, N.L., *et al* (2009). *BMC genomics*, 10:216.

Seaton, G., Haley, C. S., Knott, S. A., *et al.* (2006). *Proc of the 8th WCGALP, Brazil.*