Review of Genetics of host resistance/susceptibility to paratuberculosis

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Executive summary

This review covers the following four areas: Gene expression, Candidate gene approach, Genome wide association studies and Heritability estimates of susceptibility of livestock to Johne's disease. The science in this area has advanced considerably in the last 10 years and there is wealth of literature relating to paratuberculosis and other diseases due to mycobacteria. This review focuses almost entirely on paratuberculosis and most of it relates to cattle.

Gene expression studies have been carried out almost entirely on cattle and have used microarray technology and quantitative RT-PCRs of increasing sophistication capacity over the last 10 years. The most common tissue studied has been white blood cells or mature bovine monocyte-derived macrophages or dendritic cells from blood samples of healthy cattle or from cattle infected by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infected cattle. The normal cells were challenged with infective MAP or *Mycobacterium avium* and gene expression was studied at varying times after challenge. One study compared gene expression in ileum samples from sheep that had either paucibacillary or multibacillary Johne's disease, with samples from asymptomatic sheep. These studies showed a wide range of up-regulated and down-regulated genes in normal cells challenged by MAP or in naturally infected cells. Generally the number of upregulated genes exceeded the number of down-regulated genes in infected cells. The majority of genes of interest were involved in the inflammatory response, cell signalling pathways, apoptosis and autophagy.

The scope of these studies was somewhat confined because of the nature of the microarrays; the initial arrays (BOTL-2) were in the early phase of development and contained just over 300 spots. By 2012 the latest array (Affymetrix GeneChip® Bovine Genome Array) contained over 23,000 gene transcripts and qPCR has also advanced considerably. However, the biggest constraint is that the arrays are restricted to what genes are currently thought to be involved in various pathways and the number that can be squeezed onto a plate.

There is no literature currently published on gene expression in ruminants derived from new generation sequencing.

Candidate genes: The following candidate genes were identified in a number of studies of MAP infected animals: SLC11A1, Toll-like receptors (TLR) 1, 2 and 4, Caspase associated recruitment domain 15 (CARD15)/NOD, IL-10, Major histo-compatibility complex (MHC), interferon-gamma, EDN2, TDGF1, TGFB2, PIK3R1, *SLC39A3* (solute carrier family 39) and *TNFAIP8L1* (tumour necrosis factor alpha-induced protein 8-like protein).

Genome wide association studies have been carried out primarily in dairy cattle and identified a number of areas of interest in the cattle genome associated with paratuberculosis, but there has been little agreement between studies.

A number of heritability studies have been carried out using milk ELISA, serum ELISA and /or faecal culture, primarily in dairy cattle, but also one sheep study, and they estimated heritability of infection of 0.03 – 0.18.

Introduction

This review of the literature was carried out in fulfilment of the JDRC Contract 4.2 Control of Johne's in deer Milestone 4.2.9 Undertake a literature review of ruminant gene expression in response to MAP. The review has broadened out into a general review of the whole area of host resistance/susceptibility to paratuberculosis and is in four sections:

Gene expression
Candidate gene approach
Genome wide association studies
Heritability estimates of susceptibility of livestock to Johne's disease

The science in this area has advanced considerably in the last 10 years and there is wealth of literature relating to paratuberculosis and other diseases due to mycobacteria. This review focuses almost entirely on paratuberculosis and most of it relates to cattle.

Gene expression

Over the last 10 years a number of studies have demonstrated differences in gene expression in various tissues, including peripheral blood mononuclear cells (PBMC), ileum and mesenteric lymph nodes from animals infected with MAP compared with uninfected controls. The majority have used microarrays of various types and some have used RT-qPCR to investigate specific genes. These studies include:

- PBMC from groups of MAP infected and healthy control cows using BOTL-3 and BOTL-5 microarrays and RT-PCR (Coussens et al 2002; Aho et al 2003; Coussens et al 2003; Coussens et al 2004; Coussens et al 2005; Skovgaard et al 2006).
- An in vitro studies of gene expression in normal cattle dendritic cells incubated with MAP or *E. coli* using RT-PCR (Langelaar et al 2005).
- Differential gene expression was measured using a bovine immune-specific BOTL-5 microarray to compare the response of mature bovine monocyte-derived macrophages to co-culture with MAP and *Mycobacterium avium avium* (MAA) (Murphy et al 2006).
- Kabara et al (Kabara et al 2010) used a bovine BOTL-5 microarray to study alterations in gene expression in monocyte-derived bovine macrophages when these cells were infected with 10 different strains of MAP bacteria.
- Galindo et al (Galindo et al 2010) collected blood and spleen from hunter shot red deer infected with *Anaplasma ovis*, *Mycobacterium bovis*, or *A. ovis* and *M. bovis* and MAP, and used Affymetrix GeneChip® Bovine Genome Array microarrays to analyze gene expression profiles in PBMC for responses to these infections.
- Verschoor 2010 (Verschoor et al 2010a) used BLOPlus microarrays to compare gene expression profiles of PBMCs from healthy adult Holstein and Jersey cows that were either uninfected or sub-clinically infected with MAP.
- Smeed et al (Smeed et al 2010) used a ruminant immuno-inflammatory gene universal microarray and real-time RT-qPCR analyses to compare gene expression in ileum samples from sheep with the either paucibacillary or multibacillary Johne's disease, compared with asymptomatic sheep.

- Purdie et al (Purdie et al 2012) used Affymetrix GeneChip microarrays to study gene expression in total RNA from white blood cells from cattle taken 9, 13 and 21 weeks after experimental challenge with MAP.
- MacHugh et al. (MacHugh et al 2012) used Affymetrix GeneChip Bovine Genome Array to measure gene expression in bovine monocyte-derived macrophages (MDM) purified from seven age-matched females in response to in vitro infection with MAP at 2, 6 and 24 hours post-infection.

The early work was reviewed Pribylova et al 2009 (Pribylova et al 2009).
"Microarrays have been used frequently in the work of Coussens et al. (Coussens et al 2002; Coussens et al 2003; Coussens et al 2005) who employed them to observe the gene expression in peripheral blood mononuclear cells (PBMCs) after MAP infection. In 2002, Coussens et al. (Coussens et al 2002) found differences in gene expression profiles when comparing cows in clinical and sub-clinical phases of infection. Surprisingly, many genes were found to be repressed in cells from animals in the clinical phase, while activated in the case of animals in the sub-clinical phase. Although these results brought interesting information, the authors themselves pointed out the low number of tested samples, which could limit the validity of their conclusions. Later they were able to confirm the differences in gene expression in host cells of infected and uninfected animals. They also showed that the common response to stimulation by MAP is generally manifested by gene down-regulation in host immune cells (Coussens et al 2004). Coussens et al. (Coussens et al 2005) then affirmed their previous results concerning the different expression profiles in PBMCs infected by MAP and in control cells. Moreover, they showed an enhanced expression of transforming growth factor-β (TGF-β) and tissue inhibitors of matrix metalloproteinases (TIMPs) whose enhanced expression could lead to tissue migration deficiency of the infected PBMCs. Finally, it was shown that MAP-infected PBMCs were highly pro-apoptotic as they were expressing genes involved in apoptosis at higher levels than control cells.
Aho et al. (Aho et al 2003) also studied gene expression, but in contrast to previous articles they aimed their attention at tissues from paratuberculosis positive and negative cows. Two genes coding for tumour necrosis factor receptor associated protein 1 (TRAF1) and interleukin-1α (IL-1α), were found to be dramatically over-expressed in positive animals. Because TRAF1 is known to have an anti-apoptotic role, its increased expression could explain, according to the authors, macrophage accumulation in lesions associated with paratuberculosis. The over-expression of IL-1 and the subsequent toxicity of its protein could be connected with paratuberculosis symptoms. It is known that bovine macrophages are able to kill MAA in vitro, but do not have the ability to kill MAP (Weiss et al 2002). A comparison of macrophage expression response to MAA and MAP infection can therefore provide new information about the survival strategies of MAP and the defense mechanisms of host organism. Weiss et al. (Weiss et al 2004b) tried to solve this problem using bovine microarrays and found out a decreased ability of MAP-infected macrophages to undergo acidification, although the expression of their vacuolar H+ATPase was higher compared to those infected with MAA. As the major function of H+ATPase is related to phagosome acidification, the authors assumed this could contribute to the inability of MAP-infected macrophages to be acidified. It was also demonstrated that some genes of MAP-infected macrophages were differentially expressed in different time intervals which could be connected with the inability of the macrophages to maintain the dynamic immune response against MAP.
Subsequently, Weiss et al. (Weiss et al 2004a) used a human array to characterise the response of MAP-infected macrophages in comparison with unactivated macrophages and macrophages activated by incubation with MAA, IFN-γ or lipopolysaccharide. Lower expression of major histocompatibility (MHC) antigen class II molecule in cells infected with MAP in comparison with unactivated cells,

represented the most interesting observation. Low MHC II expression logically leads to the decreased capacity of affected macrophages to present antigens, and in the long term to a diminished immune response of the host organism. In general, many genes with various functions were differentially expressed, which gives evidence of complex interactions between macrophages and the attacking organism (Weiss et al 2004a).
The work of Murphy et al. (Murphy et al 2006) had a similar focus to that of Weiss et al. (Weiss et al 2004b, 2004a). Similarly to Weiss et al., Murphy et al. 2006 observed an increased expression of vacuolar H+ ATPase in MAP-infected macrophages. However, they observed similar expression profiles in most macrophages infected with MAA or MAP, even when some significant changes in expression were observed. Most of the differentially expressed genes belonged to or were somehow connected with three MAPK (mitogen-activated protein kinase) pathways (p38, ERK1/2 or JNK). Although MAPK pathways genes were rapidly activated in both MAA- and MAP-infected macrophages, the level of expression was considerably lower in the latter. The differences in the MAPK pathway gene expressions could be related to the observed stronger induction of gene expression in macrophages infected with MAA than with MAP."

The following is a more detailed summary of findings from the studies over the last 10 years:
(In general genes in the microarrays were considered significantly upregulated if they showed ≥ 1.5 fold change and $P < 0.05$.)

Coussens et al. (2002) found16 genes were down-regulated in clinical cows (MCRS1, FGF, Lyn B protein kinase). Eight genes from PBMCs of clinically infected cows exhibited a modest up regulation following stimulation with MAP, including CD40L, IFNγ, IL-10 and TIMP4, while PBMCs from subclinically infected cows showed significant up regulation of 11 genes, including those encoding bovine CD40L, several matrix metallo-proteinases and SPARC. In PBMCs from the subclinical cows, 16 genes were significantly down-regulated, including COX3, IL-1R1 and FGL2. Thus, the most prominent change induced by exposure of PBMCs from clinical cows to MAP in vitro tended to be repression of gene expression, while changes in similarly treated PBMCs from subclinical cows was balanced between gene activation and repression (Coussens et al 2002).

Coussens et al. (2003) concluded; (a) that T cells within PBMCs from MAP cows adopted a predominant Th 2-like phenotype (enhanced expression of IL-5, GATA 3, and possibly IL-4 mRNA), (b) that cells within infected cow PBMCs may exhibit tissue remodeling deficiencies through higher expression of tissue inhibitor of matrix metalloproteinase (TIMP) 1 and TIMP2 RNA and lower expression of matrix metalloproteinase (MMP) 14 RNA than similar cells from healthy controls, and (c) that cells within the PBMC population of M. paratuberculosis-infected cows are likely poised for rapid apoptosis (upregulation of CIDE-A, Bad, TNFRI, and Fas) (Coussens et al 2003).

Coussens et al. (2004) showed gene expression of IL-10 in PBMCs and IFN-gamma, TGF-beta, IL-5, and IL-8 in ileal tissues was increased, but IL-16 was reduced from MAP infected cattle compared with uninfected control tissues. Expression of IL-1α, IL-8, IL-2, and IL-10 mRNA was increased, but TGF-beta and IL-16 was reduced in mesenteric lymph nodes from infected cattle. Authors suggested that cells or other mechanisms capable of limiting proinflammatory responses to MAP developed in infected cattle and that a likely place for development and expansion of these cell populations were the mesenteric lymph nodes draining sites of infection (Coussens et al 2004).

Coussens et al. (2005) showed that cytokine genes LI-5, GM-CSF, Activin A receptor IB, Lymphotactin, Tie 2.0 were upregulated 2.9-5.4 fold in PBMCs from MAP infected cf control uninfected cattle. TGFb is an immune modulating cytokine that can limit pro-inflammatory responses and has profound effects on tissue remodeling via regulation of matrix metalloproteinases and their inhibitors. Tie2/Tek reduce inflammation by preventing the leakage of proinflammatory plasma proteins and leukocytes from blood vessels. Several genes encoding the MMPs and TIMPs were significantly differentially expressed in these two cell populations, including TIMPs 1, 2 and 3, MMPs 14 and 15, and ADAM17. These data are consistent with known effects of TGFb and IL-4, with TGFb known to upregulate expression of TIMP1, TIMP2, and TIMP3, and IL-4 known to upregulate TIMP2. These results are consistent with previous observations indicating that MAP has a profound effect on expression of several MMP genes, including MMP9, MMP14, and MMP23 in PBMCs from infected cows exposed to MAP in vitro (Coussens et al 2002; Coussens et al 2003). Results showed that pro-apoptosis and signal transduction gene expression was significantly upregulated for WD40 Repeat, CYCS, CIDEA and binding component 6 (Bad), but also anti-apoptosis genes MCL1 (MUSK), a 14-3-3 family member and TRAF1were also upregulated in infected cattle (Coussens et al 2005).

Langelaar et al. (2005) showed that after 6 hours of incubation of viable MAP with dendritic cells (DC) there were increases in gene expression of IL-1b (48-fold), IL-6 (9-fold), IL-10 (8-fold), IL-12 (369-fold), TNF-a (21-fold) and GM-CSF(51-fold). After 24 h of the expression of IL-10 increased from 8 to 11-fold and GM-CSF increased from 51to130-fold, while IL-1β expression declined from 48 to 3-fold, IL-6 declined from 9 to 1.3 fold, IL-12 declined from 4694 to 369-fold and TNF-α declined from 21 to 14-fold. It appeared that infection of DC by *E. coli* and MAP triggered the production of proinflammatory cytokine gene expression, dominated by IL-12, which would lead to the appropriate cell mediated immune reaction to combat infection. However, the response to MAP infection was less vigorous than to *E. coli* (Langelaar et al 2005).

Skovgaard et al 2006 demonstrated that in the BOTl-5 microarray a subset of genes in leukocytes was consistently expressed at different levels, depending upon MAP infection status. P-selectin (the SELP) and CD30L (TNFSF8) were consistently upregulated in subclinically MAP infected cattle while LIF (leukemia inhibitory factor), ADAM17 (TNFaCE), IL-1RA, Caspase-7 and ACVR2A (Activin RIIA) were inconsistently up- or down-regulated in infected Holsteins and Jersey cattle..
Three out of the 15 most significant differentially expressed genes identified in this study were proapoptotic and up regulated in cells from infected cows relative to controls, these include genes encoding CD30L (TNFSF8) (4.2-fold), IGFBP-4 (2.4-fold), and Caspase-7 (4.0-fold).

Murphy et al. 2006: 144 genes were differentially expressed in MDM cells following infection with MAA and 99 genes differentially expressed following infection with MAP. Of these genes, 37 were affected by both types of mycobacteria, with three being affected in opposite directions. Over 41% of the differentially expressed genes in MAA and MAP infected MDM cells were members of, regulated by, or regulators of the MAPK pathways. MAA was found to be a stronger activating factor than MAP. These gene expression patterns were correlated with prolonged activation of p38 MAPK and ERK1/2 by MAA, relative to MAP.

Kabara et al. 2010: Overall MAP infection altered gene expression of 78 annotated genes, regardless of strain. Large groups of apoptosis genes, transcription factors and cytokines were found to be differentially expressed in infected monocyte-derived macrophages as well as several genes not

previously linked to MAP-host interactions. The following apoptosis related genes showed changes in regulation compared with controls: CASP1 (anti-apoptosis), CASP4 (anti-apoptosis), and BCL2A1 were up-regulated, while CASP3 (pro-apoptosis), PARP1, GATA4, DFFA, BAD, TNFR1 and BCL2 were down-regulated, and CASP6 and TNFR2 were unaffected.
For most strains, IL-1α, IL-1β, IL-8, MIP3α and BCL2A1were all up-regulated, while BCL2 was down regulated. The authors commented that some late effects of Johne's disease, such as inflammatory damage to the host, may be due to drastic over-expression of IL-1 by MAP-infected intestinal macrophages (Kabara et al 2010).

Galindo et al. 2010: Four immune response genes were over-expressed in *A. ovis*/*M. bovis*/MAP-infected deer (ADAM9, CR2/CD21, FOXP1 and IL1B) and were part of the 13 genes over-expressed in *A. ovis*-infected deer. These genes included those involved in the control of innate immunity (CD21, IL1B, ATP5B, CD80, PSEN1, PTEN), adaptive immunity (ADAM9, FOXP1, AP3B1, CD5L, LCK) or both (TP53, VAV1). The results also showed that *M. bovis* infection in red deer significantly over-expressed the expression of only two genes (CD5L and TP53).

Verschoor et al. 2010: analysis identified differences in gene expression between a) healthy and infected cows, including genes involved in the inflammatory response, and calcium binding, and b) infected Holsteins and Jerseys, including genes involved in the immune response, and antigen processing and presentation. These results suggest a mixed pro- and anti-inflammatory phenotype of PBMCs from MAP-infected as compared to healthy control animals, and inherently different levels of immune and inflammatory-related gene expression between MAP-infected Holsteins and Jerseys. S100A12 (Calgranulin C), MHCII DQA, DUSP1, GNBP_11 (beta-1,3-glucan recognition protein 2), CARP (cardiac ankyrin repeat protein), Hemoglobin beta subunit (HBB), Cathepsin S, Stomatin, Platelet factor 4 (CXCL4), Fibrinogen-like 2, and S100 calcium binding protein A8 (Calgranulin A) genes were significantly upregulated in infected animals (Verschoor et al 2010a).

Smeed et al. 2010: The data showed that the two forms of disease were associated with distinct molecular profiles highlighted by the differential expression of chemokine and chemokine receptor transcripts. The cells within the lesions also showed evidence of abnormal activation; they expressed high levels of cytokine transcripts but had reduced expression levels of transcripts for T cell receptor associated molecules. Genes upregulated (1.74-2.03 fold) in PB and MB compared to asymptomatic animals: CD63, CXCR4, IGF2R, ITGB2, MMP9, TLR2, and TYROBP (DAP12).
ITGA4 and ITGAL. They were up-regulated in the paucibacillary animals, but less consistently in the multibacillary animals where the β1 integrin ITGA4 was repressed and the β2 integrins ITGAL, ITGA4 and ITGB2 were increased. TLR2 was greatly up-regulated in multibacillary lesions,
CCL2 was raised in multibacillary lesions (and 1.65-fold but $P > 0.05$ in the paucibacillary vs asymptomatic comparison), CCL5, which is associated with T cell and dendritic cell recruitment, was repressed in the paucibacillary group. IGFBP6 was differentially expressed in sheep paratuberculosis (increased in paucibacillary and repressed in multibacillary diseased ileum) (Smeed et al 2010).

Purdie et al. 2012: Blood samples from four MAP exposed and four unexposed cattle, selected based on IFNγ expressions were taken at 9, 13 and 21 weeks and RNA processed to Affymetrix GeneChip™. A stark variation was observed in expression of a number of genes along antigen presentation pathways,

suggesting that MAP exposure potentially results in the host immune response switching to a CD8 + biased antigen presentation profile.

List of downregulated genes (ave -1.5 to 7.8) exposed vs unexposed	
MHC, class II, DQ alpha 2	BOLA-DQA2
MHC class II antigen	BLA-DQB
MHC, class II, DQ alpha 5	BOLA-DQA5
Similar to guanylate binding protein 4	LOC510382
Delta/notch-like EGF repeat containing	DNER
Heat shock 70 kDa protein 1A/B	BOLA-DQB
Junctional adhesion molecule 2	JAM2
Similar to Feline leukaemia virus subgroup C receptor-related protein 2	LOC509034
Similar to guanylate binding protein 4	LOC507055
Angiogenin, ribonuclease, RNase A family, 5	ANG
Interferon-induced protein 44	IFI44
Sphingomyelin phosphodiesterase 3, neutral membrane	SMPD3
Aldehyde oxidase 1	AOX1
Minichromosome maintenance complex component 10	MCM10
Spectrin, beta, non-erythrocytic 1	SPTBN1
Lysozyme C-2	LYZ2
Hypothetical LOC509513	LOC509513
Keratin 10	KRT10
MHC, class II, DQ alpha, type 1	BOLA-DQA1
Arylsulfatase B	ARSB
Transcription factor Dp-2 (E2F dimerisation partner 2)	TFDP2
Transmembrane protein 149	TMEM149
Crystallin, mu	CRYM
Connective tissue growth factor	CTGF
Defensin, beta 4A	DEFB4A
T cell receptor, beta cluster	TRB@
Ubiquitin carboxyl-terminal esteraseL1 (ubiquitin thiolesterase)	UCHL1
Aldehyde dehydrogenase 5 family, member A1	ALDH5A1
Serpin peptidase inhibitor, clade B like	LOC511106
Neutrophil beta-defensin-9 like Peptide	BNBD-9-LIKE
Similar to Myeloid-associated differentiation marker	MGC152278
CD38 molecule	CD38
Phospholipid scramblase 4	PLSCR4
Beta-defensin 10	BNBD10
3-Hydroxy-3-methylglutaryl-Coenzyme A synthase 1(soluble)	HMGCS1
Dynactin 6	DCTN6
Similar to LOC512905 protein	LOC786352
Elongation factor RNA polymeraseII-like 3	ELL3
Uridine phosphorylase 1	UPP1
chemokine (C-X-C motif) receptor 4	CXCR4
Solute carrier family 6 member 4	SLC6A4
Parvin, beta	PARVB
Sulfiredoxin 1 homolog (S. cerevisiae)	SRXN1
Tetraspanin 7	TSPAN7
GTPase, IMAP family member 6	GIMAP6
Tachykinin 3	TAC3

List of upregulated genes (average 1.5 to 16.5) exposed vs unexposed	
Granulysin	GNLY
Killer cell immunoglobulin-like receptor, two domains	KIR2DS1
Chemokine (C-C motif) ligand 3	CCL3
MHC class I-like family A1	LOC100126815
AXL receptor tyrosine kinase	AXL
MHC, class I, A	BoLA///HLA-
MHC, class II, DRB3	BoLA-DRB3
Prostaglandin reductase 1	PTGR1
Zinc finger RNA binding protein 2	ZFR2

Brain ribonuclease	BRB
Guanine nucleotide binding protein (Gprotein), beta polypeptide 4	GNB4
Trafficking protein, kinesin binding 2	TRAK2
MHC class I antigen	BOLA-NC1
Hypothetical LOC786987	LOC786987
MHC class I heavy chain	BOLA

Excerpts from the Discussion: "Previous genomic studies suggest that in cattle, MAP modulates the host immune response to prevent inflammation (Weiss et al 2004a; Murphy et al 2006). While the bacterium is not considered to be an immune stimulating agent (Bannantine and Talaat 2010), it has been shown to inhibit macrophage processes including antigen presentation (Weiss et al 2001) and apoptosis (Kabara et al 2010). An analysis of gene expression changes in PBMC derived from adult Holstein and Jersey cattle sub-clinically positive for Johne's disease based on serum ELISA results, identified a mixed pro- and anti-inflammatory expression phenotype of differentially expressed genes in the infected versus control sample cohort and between the two breed types (Verschoor et al 2010a). In this study, we have identified a number of genes whose expression was changed in response to exposure to MAP in young cattle. Of particular interest was the finding that following exposure to MAP, the host immune response appears to be driven to enhance the expression of genes related to the ability of cells to present antigenic peptides to CD8+ T lymphocytes rather than the CD4+ pathway that is commonly associated with T helper lymphocyte responses. Our findings support the hypothesis that in bovine Johne's disease, pathogenesis may be mediated predominantly by the loss of CD4+ T lymphocyte responses during the course of the disease (Koets et al 2001). Further to this, our findings may support an additional hypothesis: at early stages post-exposure to MAP, cattle exhibiting an enhanced expression of IFNγ are driven to a MHC class I biased CD8+ T lymphocyte immune profile.

Ontological analysis revealed that some of the most significantly changed groups were related to antigen presentation. In particular, MHC classes I and II complex genes and T-cell receptor (TCR) beta genes with immunoglobulin-like constant1-set domains (IGc1) were enriched. Genes exhibiting these domains are found almost exclusively in molecules involved in the immune system and encode for highly modular proteins in which the variable and constant domains have clear, conserved sequence patterns.

In this study the MHC class II, DQ beta 1 (BLA-DQB) gene was one of the most significantly inhibited (fold change −7.8) in comparison with the control cattle cohort and several other MHC II genes were also inhibited. The inhibition of these genes could potentially affect the host's ability to present endocytosed MAP peptide fragments to CD4+ T lymphocytes.
In contrast to the MHC class II genes, expression of MHC class I genes and particularly the MHC class I heavy chain (BOLA) was greatly enhanced in the MAP-exposed cattle (fold change 16.5). The protein product of this gene belongs to the MHC Class I heavy chain prologue, a heterodimer consisting of a heavy chain and a light chain (beta-2 microglobulin). MHC Class I molecules play a central role in the immune system by presenting cytosol derived antigenic peptides to the CD8 glycoprotein and its associated TCR. MHC Class I molecules are expressed on all nucleated cells and regulate expression of a wide range of cytokines, chemokines and transcription factors.

Within this study, we present evidence that not only does exposure to MAP result in differential regulation of MHC genes, but in addition, genes associated with the TCR signalling pathway were consistently modulated across time. Expression of the TCR beta cluster (TRB@) was reduced across the three time points and an additional probe with predicted annotation of IgC T cell receptor beta

(LOC509513) showed similar expression patterns. This is a significant finding as T cells lacking expression of either the alpha or the beta subunit of the TCR do not express the TCR/CD3 complex at the cell surface. TCR signalling and ligation involves the assembly of a complex of proteins and enzymes resulting in phosphorylation and sustained signalling via the extracellular signal regulated kinase/mitogen activated protein kinase (ERK/MAPK) pathway, a process that requires several subsets of proteins including ZAP-70 tyrosine kinase. This protein is additionally utilised in signalling of other T cell expressed receptors such as CXCR4, the expression of which was consistently down regulated in the MAP-exposed cohort of animals in comparison to the unexposed control cattle. In conjunction there was the consistent inhibition of CD38 across all three sampling points. One of the functions of this transmembrane glycoprotein is the induction of phosphorylation and subsequent mobilisation of cytoplasmic calcium along the calcium signalling pathway, a mechanism that shares a functional interdependence with TCR/CD3. Taken together it is apparent that following exposure to MAP there is adaptation of genes that are associated with T cell activation both within the antigen presenting cell and the T cells themselves."
(Purdie et al 2012)

MacHugh et al. 2012: 1050, 974 and 78 differentially expressed unique genes were detected 2, 6 and 24 hour post infection (hpi), respectively. In the infected MDM the number of upregulated genes exceeded the number of downregulated genes at each time point, with the fold-change in expression for the upregulated genes markedly higher than that for the downregulated genes. Primarily genes were involved in the inflammatory response, cell signalling pathways, apoptosis and possibly autophagy. The transcriptional changes associated with cellular signalling and the inflammatory response may reflect different immuno-modulatory mechanisms that underlie host-pathogen interactions during infection.
Six genes *CCL5*, *CD40*, *CFB*, *IL1B*, *IRF1* and *TNF* were significant up-regulated and one gene (*AREGB*) was significantly down-regulated across all time points. There were a number of genes upregulated at only one time point; eg *TLR2* was the only TLR-encoding gene found to be differentially expressed (upregulated) in the infected MDM 2 hpi; a result that suggests that this PRR plays an important role in the early macrophage recognition of MAP. NF-κB-inducible cytokine and chemokine genes (*CCL20*, *CXCL2*, *IL1B*, *IL1A*, *IL6* and *TNF*), displayed the highest fold-increase in relative expression 2 hpi, suggesting endogenous macrophage cytokine and chemokine gene expression is a key early event.
Three tiers of the MAPK signalling cascade were differentially expressed in the infected MDM 2 hpi, some of which displayed opposing expression profiles. For example, *MAP3K14* (also called *NIK*) was downregulated, while *MAP3K8* was upregulated; *MAP2K3* and *MAP2K4* were both upregulated; *MAPK6* (also called *ERK3*) was upregulated and *MAPK14* (encoding a protein also designated as MAPK-p38α) was downregulated at this time point. In addition, *FOS* and *JUN* were downregulated 2 hpi, suggesting that MAPK signalling is modulated upon mycobacterial infection, a mechanism that may enable mycobacterial persistence in host macrophages.
IL10 was upregulated 2 hpi and *IL-10 signalling* was the top ranking canonical pathway at that point, supporting the hypothesis that mycobacteria exploit the anti-inflammatory activity of this cytokine to promote intracellular survival.
Several pro- and anti-apoptotic genes were differentially expressed 2 hpi and 6 hpi. These included *TNF* (upregulated, proapoptotic), *CASP1*, *CASP4*, *CASP6* (all upregulated, all proapoptotic), *BIRC3* (upregulated, anti-apoptotic) and the CASP8 and FADD-like apoptosis regulator gene (*CFLAR* upregulated - isoforms either pro- or anti-apoptotic mediators).

At the 6 hpi time point there was a notable attenuation in the fold-change upregulation of many of the inflammatory cytokine genes and the MAPK cellular signalling pathway.
Two cytosolic cell surface pathogen recognition receptors (PRRs), *TLR3* and *IFIH* were upregulated 6 hpi and they may be associated with autophagy response of MDM.
Up- and down-regulation of macrophage genes in response to MAP infection has largely abated by 24 hpi, but CD40 continued to be upregulated.(MacHugh et al 2012)

Gene expression related to *Mycobacterium bovis* in red deer

Fernandez de Mera et al. 2008: characterized the differential expression of inflammatory and immune response genes in mesenteric lymph nodes of deer naturally infected with *M. bovis* using microarray hybridization. Of the 600 ruminant inflammatory and immune response genes that were analyzed in the microarray, 157 showed X1.2-fold changes in expression in infected or uninfected deer. However, only 17 genes fulfilled the selection criteria of displaying an expression fold change
greater than 1.7 with a P-value p0.05 and were selected for further analysis. We briefly summarize the function and possible role during mycobacterial infection of the proteins encoded by these genes. **Downregulated in infected deer:** Tight junction (TJ) protein 2 (Z02) and occluding, Interleukin 2 and 11 receptors (IL-2R and IL-11R), Casein kinase II (CK2), Bactenecin (SMAP-29) and Immunoglobulin A (IgA). **Upregulated in Infected deer:** Lysosomal integral membrane protein 1 (CD63), CD62L (L-selectin/LECAM-1), Major histocompatibility complex class II (MHC-II)-associated invariant chain (CD74), Desmoglein, IgG1 and IgG2, Small subunit ribosomal RNA and High affinity IgE receptor g subunit (FcERg). As discussed above, the expression of some of the genes identified in this study, such as the IL-2R, IgGs, ribosomal RNA and CD63, have been reported previously to be affected by mycobacterial infection. However, other genes, including the IL-11R, bactenecin, CD62L, CD74, desmoglein, IgA, IgM, Z02 and occluding, constitute new findings and suggest new mechanisms by which *M. bovis* may modulate host inflammatory and immune responses. (Fernandez de Mera et al 2008)

Candidate genes for resistance/susceptibility to paratuberculosis

Purdie et al (2011) has published an extensive review entitled "Candidate gene and genome-wide association studies of Mycobacterium avium subsp. paratuberculosis infection in cattle and sheep: a review" and the section "Candidate gene approach" is presented here.

Most candidate gene discovery studies for MAP susceptibility employ a case–control design, where samples are selected from a set of cases (animals that have the disease) and controls (animals exposed but not determined to be infected). The interest is in genetic variants (alleles) or haplotypes (a linear combination of alleles) for which the frequency in cases and controls differ significantly. A range of approaches have been used to determine the significance of candidate gene variants in MAP susceptibility. One approach that is often used is an odds ratio; that is the ratio between the fraction (probability) of animals with the variant allele vs. those not having the variant in the cases vs. the controls (Bhide et al 2009). One critique of such methods is the failure in some cases to report confidence intervals for odds ratios, which would improve the interpretability of the described associations. Alternatively, genotype frequencies are tested for departure from Hardy–Weinberg equilibrium by Chi square and Fisher's exact tests to determine non-random associations between genotype frequencies in comparison to the MAP infection status of the animals (Pinedo et al 2009b). Ruiz-Larranaga et al. (Ruiz-Larranaga et al 2010a) tested statistical significance of genetic association by Chi square and correction for multiple comparisons of a particular region using permutation procedures. Association of alleles and haplotype frequency between the infected and healthy animals was determined by odds ratio analysis (Ruiz-Larranaga et al 2010a). To avoid the risk of false associations, researchers have used additional approaches. Verschoor et al. (Verschoor et al 2010b) used a combination of statistical approaches including pairwise linkage disequilibrium, principle component analysis and regression analysis by Akaike's information criterion. Another approach is to perform multivariate logistic regression for increased stringency, including variables such as breed, age or property of origin (Pinedo et al 2009a; Koets et al 2010). Reddacliff et al. (Reddacliff et al 2005) sourced animals from two separate flocks and the genotyping results were separately tested for statistical relevance, with results ranked according to allelic variability.
Occurrence of similar associations in the two individual flocks tested was considered an indicator of genetic influence on susceptibility to paratuberculosis. Similarly, Ruiz-Larranaga et al. (Ruiz-Larranaga et al 2010a) used a joint association analysis between two separate cohorts of cattle (Spanish and Dutch) to increase the statistical power of the study. For GWAS using SNP arrays, statistical approaches must be carefully chosen so as not to identify spurious associations (Pant et al 2010).

Candidate gene polymorphisms in bovine or ovine MAP susceptibility

Recently, there has been a rapid expansion in the literature in relation to candidate genes and MAP susceptibility in cattle and sheep. This has been informed in part by studies on genetic susceptibility to other mycobacterial diseases, including tuberculosis in humans, with many correlates found. These findings are collated below.

Solute carrier family 11 member 1 (SLC11A1)

The SLC11A1 gene has polymorphisms associated with MAP susceptibility in both sheep and cattle. The gene, formerly known as the natural resistance-associated macrophage protein 1 (NRAMP1) is an iron transporter protein primarily expressed in phagosomes. The protein exhibits pleiotropic effects on the early innate macrophage response to intracellular bacterial growth (Alter-Koltunoff et al 2008) including regulation of inducible nitric oxide synthase (iNOS) expression in mice (Arias et al 1997).

Several groups have looked at host cellular interactions with mycobacteria and SLC11A1. Strong genetic influences to susceptibility have been established in a mouse model of paratuberculosis (Roupie et al 2012) supported by a relationship between SLC11A1 and inducible nitric oxide synthase (iNOS), expressed at the site of infection in the gut mucosa and lymph nodes, and the pathogenesis of paratuberculosis in cattle (Delgado et al 2010).

In mice infected with Salmonella typhimurium the formation of IL-10 is suppressed via increased iNOS expression mediated by SLC11A1 (Fritsche et al 2008); this could also apply to the host response to MAP given the role of IL-10 in the pathogenesis of paratuberculosis (de Silva et al 2010).

Reddacliff et al. (Reddacliff et al 2005) identified SLC11A1 polymorphisms (Table 2) in two phenotypically defined Merino flocks with a high prevalence of Johne's disease. The animals were classified as severe, mild or non-diseased based on a combination of clinical signs, serological (agar-gel immunodiffusion test) and histopathological analysis, BACTEC faecal and tissue culture and intradermal testing for delayed type hypersensitivity (Table 3). The microsatellite markers were chosen based on previously published research in which associations had been reported or polymorphisms within a gene family were implicated in susceptibility to mycobacterial diseases such as tuberculosis, leprosy and M. avium infections in humans. Samples were sourced from two separate flocks (Table 3) and the genotyping results were separately tested for statistical relevance. Associations were thought likely if they were found to be independently significant in both flocks. In a similar study on goats, paratuberculosis susceptibility was significantly associated with a microsatellite in the 3' untranslated region (3'-UTR) of SLC11A1, which may be related to the levels of the gene expressed in response to MAP exposure (Korou et al 2010).

SLC11A1 polymorphisms in cattle were first described by Pinedo et al. (Pinedo et al 2009a). Microsatellite markers were used to address polymorphisms of three candidate genes (SLC11A1, interferon-gamma IFN-γ and TLR4) as risk factors for MAP infection in Holstein, Jersey and Brahman-Angus cross (total 431 cattle), obtained from five dairy herds in the USA (Table 3). MAP prevalence at the time of sampling was unknown although all herds had a history of clinical MAP infections. An animal was classified as MAP positive if it tested positive in any single diagnostic procedure whereas a MAP negative classification required that an animal test negative for all five diagnostic tests (Table 3). Microsatellite markers tested in this study were chosen based on previous studies, including that of Reddacliff et al (Reddacliff et al 2005). There were significant differences in the allelic frequencies of two SLC11A1 alleles between MAP positive and negative cattle, even when breed and age were included in the logistic regression model (Table 2).

In an attempt to analyse the entire functional variability of the SLC11A1 gene in cattle, including all exons and flanking intronic, untranslated and promoter regions, Ruiz-Larranaga et al. (Ruiz-Larranaga et al 2010b) carried out a SNP-based candidate gene study. Initially 57 SLC11A1 SNP

Tables 2 and 3 copied from Reddacliff et al. (Reddacliff et al 2005) but reference numbers invalid.

Table 2
Statistically significant candidate gene polymorphisms associated with paratuberculosis susceptibility.

Gene	Species	Micro-satellite	SNP	Mutation[a]	Location	Significance[b]	Reference	Year
CARD15	Cattle	–	2197T>C	Cys733Arg	LRR[c]	2.32 (1.41–3.83)[d,e]	Pinedo et al. [39] Pinedo et al. [59]	2009
	Cattle	–	1908C>T	–	3′-UTR[c]	2.043 (1.22–3.42)[f]	Ruiz-Larranaga et al. [73]	2010
IFNγ	Cattle	–	2781G/T	Gly134Val	Exon 1	1.98 (1.11–3.51)[d,g]	Pinedo et al. [37]	2009
IL10RA	Cattle	–	984G>A	Syn [c]	Coding	2.27 (1.4–3.67)[h]	Verschoor et al. [38]	2010
	Cattle	–	1098C>T	Syn	Coding	2.27 (1.4–3.67)[h]	Verschoor et al. [38]	2010
	Cattle	–	1269T>C	Syn	Coding	2.27 (1.4–3.67)[h]	Verschoor et al. [38]	2010
	Cattle	–	1302A>G	Syn	Coding	2.27 (1.4–3.67)[h]	Verschoor et al. [38]	2010
MHC	Sheep	163/*	–	–	–	$p < 0.05$[i]	Reddacliff et al. [11]	2005
SLC11A1	Sheep	162/*	–	–	Intron 1	$p < 0.05$[i]	Reddacliff et al. [11]	2005
	Cattle	275/*	–	–	3′-UTR	2.01 (1.08–3.76)[e]	Pinedo et al. [37]	2009
	Cattle	279/*	–	–	3′-UTR	5.31 (1.56–17.9)[e]	Pinedo et al. [37]	2009
	Cattle	–	1067C>G	Pro356Ala	Transmembrane domain 8	1.484 (1.049–2.099)	Ruiz-Larranaga et al. [34]	2010
	Cattle	–	1157-91A>T	–	Intron 11–12	1.592 (1.095–2.314)	Ruiz-Larranaga et al. [34]	2010
TLR1	Cattle	–	G658A	Val220Met	Ectodomain	3.459	Mucha et al. [57]	2009
	Sheep	–	448A>G	Ser150Gly	Ectodomain	9.08[j]	Bhide et al.	2007
	Sheep	–	517G>Y	Glu173[Lys,Glu]	Coding	9.08[k]	Bhide et al.	2007
	Sheep	–	658A>G	Val220Met	Ectodomain	9.08[j]	Bhide et al.	2007
TLR2	Cattle	–	2038A>G	Ile680Val	Toll/IL-1R domain	NA[l]	Mucha et al. [57]	2009
	Cattle	–	1903T>C	Syn	Putative LRR	1.7 (1.2–2.8)[h]	Koets et al. [40]	2010
	Sheep	–	2008A>Y	Phe670Leu	Toll/IL-1R domain	4.5[k]	Bhide et al.	2007
	Sheep	–	2037T>C	Leu679Phe	Toll/IL-1R domain	2.01[k]	Bhide et al.	2007
TLR4	Cattle	–	892G>Y	Gly298[Arg,Trp]	Ectodomain	NA[l]	Mucha et al. [57]	2009
	Cattle	–	895G>A	Asp299Asn	Ectodomain	NA[l]	Mucha et al. [57]	2009
	Cattle	–	1165G>A	Gly389Ser	Ectodomain	NA[l]	Mucha et al. [57]	2009
	Cattle	–	1167T>C	Gly389Ser	Ectodomain	NA[l]	Mucha et al. [57]	2009
	Sheep	–	1066T>C	Phe356Leu	Ectodomain	1.64[j]	Bhide et al.	2007

[a] Change in the amino acid sequence of the protein, if the polymorphism is within the translated region.
[b] Significance presented as odds ratio (95% confidence interval) or p value for Chi-square/Fisher exact test.
[c] Abbreviations: LRR, leucine rich repeats; 3′-UTR, 3′ untranslated region; Syn, synonymous, no amino acid change.
[d] Odds ratio determined by univariate, ordinal (0 = homozygous, 1 = heterozygous mutant, 2 = homozygous mutant) analysis.
[e] Effect was also significant using multivariate analysis, considering factors such as breed and age.
[f] Significance for the Spanish population of Holstein-Friesian only.
[g] Significance was lost on multivariate analysis, considering factors such as breed and age.
[h] Odds ratio for the dominance effect.
[i] Significance of the association with severe disease.
[j] Odds ratio for homozygous mutant.
[k] Odds ratio for heterozygous mutant.
[l] Not applicable as 100% (14/14) of cattle with these mutations all had a disease phenotype.

were identified among 14 bovine breeds; 24 were novel, previously unidentified SNP. The SNP were validated in Holstein-Friesians and 24 were selected for association studies. Two independent subpopulations of Holstein-Friesian cattle were studied; 33 Spanish herds tested once and 8 Dutch farms that had undergone repeat testing (Table 3). MAP infection status was classified as in the study of Pinedo et al. (Pinedo et al 2009a), with positive status assigned if an animal was positive in any individual test. Since paratuberculosis has been present in European herds since 1895, the study assumed that exposure to MAP infection is prevalent in all herds. Although differential significance of SLC11A1 polymorphisms was found between the two cohorts, a joint association analysis combining the populations also identified two minor allele frequency SNP that were significantly associated with susceptibility to paratuberculosis (Table 3). One of the identified SNP, which leads to an amino acid substitution in the highly conserved transmembrane region 8 of SLC11A1, could be associated with

altered stability or secondary structure of the molecule and thus functional impairment (Ruiz-Larranaga et al 2010b).

Table 3
Sample numbers and classification methods.

Animal/breed	MAP positive	MAP negative	Reference	Year	Test used
Sheep					
Merino (flock A)	75	31	Reddacliff et al. [11]	2005	Faecal culture, Clinical signs, Histo[a], AGID[b], DTH[b]
Merino (flock B)	42	43	Reddacliff et al. [11]	2005	Faecal culture, Clinical signs, Histo[a], AGID[b], DTH[b]
Tsigai	82	838	Bhide et al.	2007	Clinical signs, ELISA[c] (Pourqier), PCR (blood)[d]
Cattle					
Holstein	73	158	Gonda et al.	2007	Faecal culture, ELISA (IDEXX)
Holstein/Jersey/Brahman-Angus[e]	126	305	Pinedo et al. [39]	2009	Faecal culture, ELISA, PCR[f] (Histo,PCR on subset)
Holstein/Jersey/Brahman-Angus[e]	126	305	Pinedo et al. [59]	2009	Faecal culture, ELISA, PCR[f] (Histo,PCR on subset)
Holstein/Jersey/Brahman-Angus[e]	126	305	Pinedo et al. [37]	2009	Faecal culture, ELISA, PCR[f] (Histo,PCR on subset)
Holstein	90[g]	128[g]	Settles et al. [83]	2009	Faecal culture, tissue culture (ileum and assoc. nodes)
Slovak spotted	1	9	Mucha et al. [57]	2009	Clinical signs, PCR (blood)
Slovak spotted × Holstein	0	16	Mucha et al. [57]	2009	Clinical signs
Polish red	5	59	Mucha et al. [57]	2009	Clinical signs
Holstein	27	109	Mucha et al. [57]	2009	Clinical signs
Pinzgauer	0	247	Mucha et al. [57]	2009	Clinical signs
Slovak Simmental	103	103	Mucha et al. [57]	2009	Clinical signs
Dark brown Carpathians	24	8	Mucha et al. [57]	2009	Clinical signs
Holstein-Friesian (case control)	12	12	Koets et al. [40]	2010	Faecal culture, Histo, Clinical signs
ND[h] (Confirmation study)	245	308	Koets et al. [40]	2010	Faecal culture, ELISA (Pourqier)
ND (Immune function study)	50	0	Koets et al. [40]	2010	Faecal culture, ELISA (Pourqier)
Holstein-Friesian (Spain)	129	114	Ruiz-Larranaga et al. [34]	2010	Faecal culture, PCR (faecal) ELISA
Holstein-Friesian (Netherlands)	138	177	Ruiz-Larranaga et al. [34]	2010	Faecal culture, ELISA
Holstein	90	142	Pant et al. [41]	2010	ELISA (HerdChek IDEXX) or milk ELISA
Holstein	204	242	Verschoor et al. [38]	2010	ELISA (HerdChek IDEXX) or milk ELISA
Holstein-Friesian (Spain)	127	114	Ruiz-Larranaga et al. [73]	2010	Faecal culture, PCR (faecal), ELISA
Holstein-Friesian (Netherlands)	178	226	Ruiz-Larranaga et al. [73]	2010	Faecal culture, ELISA
Holstein	90[g]	128[g]	Zanella et al. [85] Neibergs et al. [84]	2010	Faecal culture, tissue culture (ileum and assoc. nodes)
Holstein (case control study)	483	483	Minozzi et al. [88]	2010	ELISA (ID-screen)
Holstein (confirmation study)	140	137	Minozzi et al. [88]	2010	ELISA (ID-screen)
Holstein	521	1025[i]	Kirkpatrick et al. [86]	2010	Faecal culture, ELISA (Shin et al. 2008)

[a] Histopathology on tissues after necropsy.
[b] AGID: agar-gel immunodiffussion test, DTH: delayed-type hypersensitivity.
[c] ELISA refers to serum/plasma ELISA, with type in brackets if specified in the article.
[d] *IS900* PCR, with the sample type specifed in brackets.
[e] Holstein $n = 299$, Jersey $n = 50$ and Brahman-Angus cross $n = 82$.
[f] *IS900* PCR on milk, blood and faeces performed. Subset were tested by *IS900* PCR of tissues at necropsy.
[g] Defined by tissue culture. Alternate analyses conducted based on tissue and faecal culture results.
[h] ND: Not defined.
[i] These were used to develop a multi-marker model, otherwise the number of MAP negative controls used was not defined.

Toll-like receptors

The TLR are a family of transmembrane signaling molecules that bind to conserved pathogen associated molecular patterns (PAMP) and are intrinsically involved in triggering both the innate and the adaptive immune response mechanisms (Takeda et al 2003). TLRs are found in all animal species and although most mammalian species share up to eleven TLR genes there are variations both across and within species (McGuire et al 2006; Jann et al 2008; Chang et al 2009; Jann et al 2009). TLRs 1, 2 and 4 have been implicated in cellular recognition of mycobacteria, binding cell wall components including lipoproteins (Quesniaux et al 2004; Delbridge and O'Riordan 2007). TLR-2 in particular is involved in the early recognition of intestinal pathogens including mycobacteria (Weiss et al 2008).
In 2009 Mucha et al. published data suggesting a link between TLR 1, 2 and 4 polymorphisms and paratuberculosis susceptibility of several breeds of cattle (Mucha et al 2009). The study sourced 711 cattle of various breeds from three Eastern Slovakian farms which had an historical high within-herd prevalence of MAP (7–10%). Initial selection of animals was based on evidence of clinical signs

(weight loss and/or chronic diarrhoea) with additional 4–5 apparently healthy animals that had close contact with suspected clinical cases included into the trial. However, IS900 blood DNA nested PCR alone was used to definitively classify the animals as MAP positive (n = 160) or negative (n = 551). The greatest proportion of MAP positive animals was found in the Slovakian Simmental cattle population (Table 3). To identify polymorphisms, primers were designed to amplify regions of TLR1 covering leucine rich repeat (LRR) ectodomain and primers for TLR2 and TLR4 genes were constructed in regions of previously reported polymorphisms. PCR single-stranded polymorphism analysis identified putative polymorphisms, representative samples of which were sequenced and associations between polymorphisms and MAP infection assessed. The results suggested a number of breed-dependent TLR polymorphisms of which several were indicative of susceptibility-based linkage. The majority of the animals (95%) displayed one of two TLR1 genotypes (GenBank ID EU532011.1) regardless of breed, which is unsurprising as this sequence is strongly conserved
in both Bos taurus and Bos indicus. Linkage analysis to identify mutations within the genotypes and susceptibility to MAP infection revealed that 85.7% (n = 18) of cattle carrying a TLR1 Val220Met mutation were MAP positive, but it is not clear to which genotype this mutation pertained. There were five TLR2 genotypes; these displayed significant breed-dependent distribution. Non-synonymous or mis-sense mutations involving an amino acid change in the
TLR2 gene were reported in 20% (n = 32) of MAP infected cattle. Cattle in a further infected group (n = 14) all possessed a mutation in TLR2 and four mutations in the TLR4 gene (Table 2), though over thirty other identified missense mutations had no significant effect on susceptibility.

A similar study looking for TLR polymorphisms in relation to MAP infected sheep was published by the same group using similar design of primers, experimental methodology and analysis (Bhide et al 2009). A total of 920 Tsigai sheep were obtained from four farms with high MAP prevalence in eastern Slovakia. Experimental classification of MAP status was determined by Pourquier ELISA and blood DNA IS900 nested PCR (Table 3). Novel polymorphisms were found in ovine TLR1, 2 and 4 genes. Six SNP were identified that gave an odd's ratio indicative of an association with increased susceptibility to MAP infection (Table 2).

Pinedo et al. (Pinedo et al 2009a) reported no correlations between MAP susceptibility and three TLR4 polymorphisms in a cohort of 431 cattle (Table 3). The three TLR4 SNP sequences used in this publication and a further publication (Pinedo et al 2009c) were sourced from a previous bovine study in which 32 TLR4 SNP were found across 20 haplotypes (White et al 2003). There is no reported haplotype linkage between the SNP and there is also no apparent overlap between polymorphism/mutation locations in either this or the Mucha et al. (2009) study.

Recently, a comprehensive study utilised SNP analysis to seek potential genome variation of TLR2 genes in bovine paratuberculosis (Koets et al 2010). Holstein Friesian cattle from twelve Dutch farms were classified as MAP positive (n = 12), based on faecal culture evidence of shedding, clinical signs and histopathology, or age-matched MAP negative herd-mates (n = 12) (Table 3). Five primers were designed to encompass the coding sequence of the TLR2 gene, located on bovine chromosome 17 (Bos taurus autosome (BTA)17). Analysis of the sequenced PCR products revealed 82 polymorphisms in 21 unique positions in the coding sequence, with five SNP frequently seen in the MAP negative group. Of these, the SNP TLR2-1903T>C mutation had significant allelic association with the MAP positive group (Table 2). These finding were validated by sequencing analyses of 553 adult cattle sourced from eight additional farms with endemic paratuberculosis (average prevalence 32%). These animals were categorised MAP positive (n = 245) or MAP negative (n = 308) based on collated faecal culture and serum ELISA results from testing carried out over five to seven consecutive sampling periods as part of the Dutch Animal Health survey. This confirmed the statistical association with paratuberculosis

susceptibility of the SNP identified in the earlier trial. Cells transfected with the TLR2 variants showed a functional correlate between susceptibility and the response to TLR2 agonists. Proliferation studies on a further 50 MAP positive cattle from 27 supplementary farms confirmed that the 'resistant' phenotype gave a functional advantage in response to specific mycobacterial antigen. The findings from this study revealed a significant association between the presence of the TLR2−1903T>C mutation and susceptibility to paratuberculosis.

Together these studies highlight a link between TLR2 and resistance to MAP infections. This is consistent with the known functional role of TLR2 in the recognition of MAP (Ferwerda et al 2007) and at the level of gene expression, the significant up regulation of the TLR2 gene at sites of MAP infection in sheep (Nalubamba et al 2008; Taylor et al 2008). As TLR2 forms heterodimers with TLR1 and TLR6 in order to recognise pathogens (Wetzler 2003), there might also be susceptible genotypes associated with TLR1 mutations (reported above) and potentially TLR6. In support of this hypothesis, there is increased expression of TLR1 and TLR6
in animals with paratuberculosis (Plain et al 2010).

Caspase associated recruitment domain 15 (CARD15)/NOD2

The nucleotide oligomerisation domain (NOD) proteins such as CARD15 are pattern recognition receptors. The ligand for CARD15 is a muramyl dipeptide derived from bacterial peptidoglycan (Girardin et al 2003). Formerly known as NOD2, it is highly expressed in monocytes and epithelial cells and is composed of three segments: aNH2terminal caspase recruitment domain, a nuclear binding domain and a LRR domain. The LRR domain is capable of recognising cell wall
constituents of mycobacteria and inducing cytokine production by triggering transcription factors such as nuclear factor kappa beta (NFκB) (Abbott et al 2004). The CARD15 protein is found
in the inflamed gut of Crohn's disease patients and polymorphisms within the LRR domain have been associated with Crohn's disease susceptibility (Ogura et al 2001). Patients homozygous
for the most common polymorphism in the CARD15 gene show a decreased expression of IL-10 in peripheral blood mononuclear cells following stimulation with TLR2 (Netea et al 2004; Maeda et al 2005).

Pinedo et al. (Pinedo et al 2009c) published a study of candidate haplotype-based association analysis on 431 paratuberculosis exposed cattle (Holstein, Jersey and Brahman-Angus crosses). This was the same cohort as described in studies of SLC11A1 (Table 3). Two bovine CARD15 SNP were selected based on results from a previous study (Taylor et al 2006). Various statistical
analysis methods were applied resulting in prediction of significance of the SNP 2197T>C in relation to paratuberculosis susceptibility (Table 2). This SNP is situated in the LRR domain and is responsible for an amino-acid substitution. A second paper utilising the same cohort sought
further association between the CARD15 gene polymorphisms by the inclusion of one additional SNP in CARD15 (Pinedo et al 2009b). A significant association between infection and the
variant allele was confirmed for SNP 2197T>C. A significant breed effect was reported for this SNP, with the highest proportion of the variant allele found in the Brahman-Angus population.

The association between CARD15 polymorphisms and MAP susceptibility has also been tested in the Holstein- Friesian breed (Ruiz-Larranaga et al 2010a). These are Bos taurus, as opposed to the Brahman-Angus which is a Bos taurus×Bos indicus mixed breed, and are relevant as the majority of breeds important for intensive meat and milk production are Bos taurus. Interestingly, the CARD15 SNP linked to MAP susceptibility by Pinedo et al. (Pinedo et al 2009b; Pinedo et al 2009c) (2197T>C) is reported to be monomorphic (have only one major allele in ≥99% of the population) rather than polymorphic for this breed (Taylor et al 2006).

Ruiz-Larranaga et al. (Ruiz-Larranaga et al 2010a) carried out a candidate gene study using 18 SNP spanning exons, introns and the 3'-UTR of the CARD15 gene. The SNP were compiled from previous reports as well as in silico analysis of the bovine CARD15 gene sequence. The sample cohort were Holstein-Friesian cattle used previously for studies by the same group on SCL11A1 (described above), sourced from 33 Spanish or 8 Dutch herds (Table 3). Initially the Spanish cohort was genotyped and 14 of the 18 SNP were identified as monomorphic. The remaining polymorphic SNP (C.2634-459G>A, C.3020A>T, C.*1458C>A and C.*1908C>T)
were tested in the Dutch sample cohort and allele variations were detected. All four polymorphic SNP were common in both the Spanish and the Dutch samples and fit Hardy–Weinberg equilibrium; they showed pairwise linkage disequilibrium and formed a haplotype block in
both populations. A MAP susceptibility association was suggested for the C allele of SNP 1908C>T within the Spanish sample cohort however this effect was not present in the Dutch cattle. This SNP is located within the last 10 nucleotides of the 3'-UTR region of the CARD15 gene where there is little evidence of sequence conservation between species. The authors suggested that the SNP is not directly involved in phenotypic variation however they suggest that
it may be in linkage disequilibrium with a causative polymorphism.

Interleukin 10 receptor alpha

IL-10 is a cytokine that primarily acts as a negative feedback mechanism for T lymphocytes and is as an essential immunoregulator in bacterial infection. From the perspective of MAP infection, IL-10 prevents excessive T helper 1 and CD8+ T lymphocyte responses that may lead to immunopathology associated with infection. The cytokine also prevents overproduction of interleukins 4, 5 and 13. The IL-10 receptor alpha (IL-10Rα) gene encodes a ligand-binding subunit of the IL-10R and therefore is a determinant of IL-10 responsiveness. As previously discussed, IL-10 production may be limited via increased iNOS expression in a mechanism that is mediated by another potential MAP susceptibility related gene, SLC11A1.
IL-10Rα polymorphisms have been associated with bovine MAP infection status (Verschoor et al 2010b). Verschoor et al. (2010) conducted a candidate gene-based study of MAP susceptibility sourcing Holstein cattle from six commercial farms in Ontario with a history of high MAP prevalence. The infection status was determined by ELISA, with 204 MAP positive and 242 healthy negative cattle included in the study (Table 2). SNP discovery was performed for IL-10 and its receptor subunits (IL-10Rα and IL-10Rβ), transforming growth factor beta (TGF_1) and its two receptors (TGFBR1 and TGFBR2) and SLC11A1.SNPgenotyping revealed tightly
linked groups within the two sets of IL-10R related SNP.
Further haplotype analysis was carried out on IL-10Rα related SNP only. Although a number of SNP were revealed for each gene only four tightly linked SNP related to IL-10Rα (984G>A, 1098C>T, 1269T>C and 1302A>G) showed statistically significant association with MAP infection, with a strong additive and dominance relationship at the GCTA allele. Cattle with these polymorphisms had a higher probability of MAP infection. None of the SNP from the other
genes tested demonstrated an association with MAP susceptibility in this study.
Although previous studies have correlated the action of IL-10 to the pathways of other susceptibility related candidate genes (such as SLA11IA), this is the first evidence of a susceptibility correlation with the IL-10 gene itself.

Major histocompatibility complex and interferon-gamma

MHC genes are involved in antigen presentation to T cells resulting in IFN-γ that in turn leads to macrophage activation, nitric oxide production and MHC class II expression. There is down regulation of MHC expression and thus antigen presentation in bovine macrophages infected with MAP compared to macrophages infected with nonpathogenic *M. avium* (Weiss et al 2001), similar to other pathogenic mycobacteria (Baena and Porcelli 2009). A Th1 response associated with the production of IFN-γ is considered protective against intracellular bacteria and is associated with MAP infection (Begg et al 2009). Susceptibility in humans to mycobacterial diseases has been associated with mutations in IFN-γ receptor genes (Doffinger et al 2000; van de Vosse et al 2009). Thus IFN-γ and MHC genes are key targets for genetic association studies.
Reddacliff et al. (Reddacliff et al 2005) reported an association of MHC polymorphisms with susceptibility to MAP infection. The study design involving two Merino sheep flocks has been described above in relation to identified SLC11A1 polymorphisms. One genotype (163/*) was associated with the presence of disease and/or clinical signs in both flocks tested. Pinedo et al. (Pinedo et al 2009a) examined the association with MAP of two previously reported SNP in the bovine IFN-γ gene (Schmidt et al 2002). The same cohort of Holstein, Jersey and Brahman-Angus cross cattle from five dairy herds with a history of clinical MAP was examined as for the SLC11A1 association study (Table 3). A significant association with infection was found for SNP 2781G>T, however the significance was lost when a multivariate analysis was performed to control for variables including breed and age (Pinedo et al 2009a) (Table 2). Alleles of both MHC and IFN-γ have also been shown to be associated with increased blood IFN-γ responses following paratuberculosis vaccination of Merino sheep, providing further evidence for a role of genetic polymorphisms in these genes in protective immune responses to MAP infection (Dukkipati et al 2010).

Similar candidate gene review

A similar review published recently (Kirkpatrick 2010) also listed SLC11A1, NOD2/CARD15, TLR1, 2 and 4 and IFN-γ as significant candidate genes associated with paratuberculosis susceptibility that had been demonstrated by the same authors as Purdie et al.

Additional candidate genes identified using microarrays

There have been a number of recent studies of genes associated with MAP tissue infection, fecal shedding or disease in cattle. Using the Illumina Bovine SNP50 BeadChip, Neibergs et al (Neibergs et al 2010) identified four genes, EDN2, TDGF1, TGFB2, and PIK3R1, which produce proteins with known functions that may be of relevance to MAP tissue infection or Johne's disease. The following summaries provided by NCBI (http://www.ncbi.nlm.nih.gov):

EDN2 (endothelin 1). This gene encodes a member of the endothelin protein family of secretory vasoconstrictive peptides. The preproprotein is processed to a short mature form which functions as a ligand for the endothelin receptors that initiate intracellular signaling events. This gene product is involved in a wide range of biological processes, such as hypertension and ovulation. [provided by RefSeq, Oct 2008]

TDGF1 (teratocarcinoma-derived growth factor 1). This gene encodes an epidermal growth factor-related protein that contains a cripto, FRL-1, and cryptic domain. The encoded protein is an

extracellular, membrane-bound signaling protein that plays an essential role in embryonic development and tumor growth. Mutations in this gene are associated with forebrain defects. Pseudogenes of this gene are found on chromosomes 2, 3, 6, 8, 19 and X. Alternate splicing results in multiple transcript variants. [provided by RefSeq, Mar 2010]

TGFB2 (transforming growth factor beta 1 induced transcript 1). This gene encodes a member of the transforming growth factor beta (TGFB) family of cytokines, which are multifunctional peptides that regulate proliferation, differentiation, adhesion, migration, and other functions in many cell types by transducing their signal through combinations of transmembrane type I and type II receptors (TGFBR1 and TGFBR2) and their downstream effectors, the SMAD proteins. Disruption of the TGFB/SMAD pathway has been implicated in a variety of human cancers. The encoded protein is secreted and has suppressive effects of interleukin-2 dependent T-cell growth. Translocation t(1;7)(q41;p21) between this gene and HDAC9 is associated with Peters' anomaly, a congenital defect of the anterior chamber of the eye. The knockout mice lacking this gene show perinatal mortality and a wide range of developmental, including cardiac, defects. Alternatively spliced transcript variants encoding different isoforms have been identified. [provided by RefSeq, Sep 2010]

PIK3R1 phosphoinositide-3-kinase, regulatory subunit 1 (alpha). Phosphatidylinositol 3-kinase phosphorylates the inositol ring of phosphatidylinositol at the 3-prime position. The enzyme comprises a 110 kD catalytic subunit and a regulatory subunit of either 85, 55, or 50 kD. This gene encodes the 85 kD regulatory subunit. Phosphatidylinositol 3-kinase plays an important role in the metabolic actions of insulin, and a mutation in this gene has been associated with insulin resistance. Alternative splicing of this gene results in four transcript variants encoding different isoforms. [provided by RefSeq, Jun 2011]

Pant et al (Pant et al 2010) identified a number of genes associated with resistance to paratuberculosis in cattle, including:
SLC39A3 (solute carrier family 39), about which little is known other than it is a zinc influx transporter, but may have a role in reducing inflammation and restoring T cell function (Kahmann et al 2008).
TNFAIP8L1 (tumour necrosis factor alpha-induced protein 8-like protein). The TNF-alpha-induced protein 8 family were recently identified as important for maintaining immune homeostasis (Lou and Liu 2011).

Genes associated with Tb in the Ingenuity Pathway Analysis (IPA)

A number of genes from the JDRS gene expression study are listed in IPA as associated with tuberculosis (Tb). These include SLC11A1 (NRAMP), IL8 and CD209.

SLC11A1 (solute carrier family 11, A1) gene: This gene is a member of the solute carrier family 11 (proton-coupled divalent metal ion transporters) family and encodes a multi-pass membrane protein. The protein functions as a divalent transition metal (iron and manganese) transporter involved in iron metabolism and host resistance to certain pathogens. Mutations in this gene have been associated with susceptibility to infectious diseases such as tuberculosis and leprosy, and inflammatory diseases such as rheumatoid arthritis and Crohn disease. Alternatively spliced variants that encode different protein isoforms have been described but the full-length nature of only one has been determined. [provided by RefSeq, Jul 2008]. It is primarily expressed in phagosomes and functions as a divalent transition metal

(iron and manganese) transporter involved in iron metabolism and host resistance to certain pathogens. (Also see above)

IL8 gene: The protein encoded by this gene is a member of the CXC chemokine family and is one of the major mediators of the inflammatory response. It functions as a chemoattractant, and its role in the cell includes chemotaxis, activation, migration, recruitment, adhesion, chemoattraction, attraction, transendothelial migration, binding, stimulation and it is also a potent angiogenic factor. This gene and other ten members of the CXC chemokine gene family form a chemokine gene cluster. It regulates: CXCR2, CXCR1, IL8 Receptor, ITGB2, MMP9, Erk1/2, ITGAM, Ca2+, Mapk, MAPK3, Erk, Akt, calcium, ITGAL. It is regulated by: TNF, lipopolysaccharide, IL1B, NFkB, IFNG, SB203580, poly rI:rC-RNA, IL1A, TLR4, phorbol myristate acetate, PD98059, NFKBIA, P38 MAPK, Flagellin, U0126. It binds: CXCR1, CXCR2, RELA, Ap1, AP-1, NFkB, DARC, NFKB1, RNA polymerase II, CEBPB, Histone h3, FOS, JUN, HDAC1n (GeneView). A quantitative immunoassay revealed that IL-8 protein release was significantly elevated in supernatants of macrophages and in lavage fluid obtained from patients with pulmonary tuberculosis compared to normal controls (Zhang et al 1995).

CD209 gene: This gene encodes a transmembrane receptor and is often referred to as DC-SIGN because of its expression on the surface of dendritic cells and macrophages. The encoded protein is involved in the innate immune system and recognizes numerous evolutionarily divergent pathogens ranging from parasites to viruses with a large impact on public health. Molecular functions include receptor activity, sugar binding, mannose binding, peptide antigen binding, virion binding and metal ion binding. Biological processes include endocytosis, cell adhesion, heterophilic cell-cell adhesion, leukocyte cell-cell adhesion, cell-cell recognition, virus-host interaction, virion attachment to host cell surface receptor, viral genome replication, antigen processing and presentation, intracellular signal transduction, interspecies interaction between organisms, innate immune response, intracellular virion transport and peptide antigen transport. Associated with Tb and M.bovis infection (Entrez Gene).

Genes associated with inflammation:

A number of genes from the JDRS gene expression study code for proinflammatory cytokines and immune mediators, including C-C and C-X-C motif chemokines and receptors, interferon family proteins, tumour necrosis (TNF) superfamily members and interleukins IL1A and IL8.

The IFIT (interferon-induced protein with tetratricopeptide repeats) gene family: the members of this family, which includes IFIT2, function to restrict intracellular pathogens and virus infection through alteration of cellular protein synthesis. IFIT2 is expressed after virus infection directly upon IRF-3 activation as well as upon IFN signaling because of the presence of both IRF-3 and ISGF3 binding sites in the *Ifit2* promoter. It has been shown that IFN-induced IKKε signaling pathway of specific STAT1 phosphorylation and IFIT2 expression imparts innate antiviral immunity to restrict West Nile Virus infection and control viral pathogenesis. (Perwitasari et al 2011).

CXCL14 gene: this gene codes for a cytokine involved in the homeostasis of monocyte-derived macrophages rather than in inflammation. It is chemotactic for monocytes and can activate these cells in the presence of an inflammatory mediator called prostaglandin-E2 (PGE2). It is also a potent chemoattractant and activator of dendritic cells, is implicated in homing of these cells, and can stimulate the migration of activated NK cells. It inhibits angiogenesis, possibly as a result of its ability to block endothelial cell chemotaxis (RefSeq).

CXCL2 gene: Chemokine (C-X-C motif) ligand 2 is a small cytokine belonging to the CXC chemokine family that is also called macrophage inflammatory protein 2-alpha (MIP2-alpha), Growth-regulated protein beta (Gro-beta) and Gro oncogene-2 (Gro-2). CXCL2 is 90% identical in amino acid sequence as a related chemokine, CXCL1. This chemokine is secreted by monocytes and macrophages and is chemotactic for polymorphonuclear leukocytes and hematopoietic stem cells. The gene for CXCL2 is located on human chromosome 4 in a cluster of other CXC chemokines (RefSeq).

CXCL5 gene: The protein encoded by this gene is an inflammatory chemokine involved in neutrophil activation and is produced concomitantly with interleukin-8 (IL8) in response to stimulation with either IL1 or TNFA. It is produced following stimulation of cells with the inflammatory cytokines interleukin-1 or tumor necrosis factor-alpha (RefSeq).

IL17F gene: codes for a proinflammatory cytokine similar to IL17 that responds to the invasion of the immune system by extracellular pathogens and induces destruction of the pathogen's cellular matrix. IL17 acts synergistically with TNF and IL1. It is expressed by activated T cells induced by IL23, which can result in destructive tissue damage in delayed-type reactions. It has been shown to stimulate the production of several other cytokines, including IL6, IL8, and CSF2/GM-CSF. This cytokine is also found to inhibit the angiogenesis of endothelial cells and induce endothelial cells to produce IL2, TGFB1/TGFB, and monocyte chemoattractant protein-1 (RefSeq).

CCL3/MIP-1 α gene: Macrophage inflammatory protein-1 (MIP-1), MIP-1α (aka CCL3) and MIP-1β (aka CCL4) are chemokines crucial for immune responses towards infection and inflammation. In humans, there are two major forms, MIP-1α and MIP-1β, produced by macrophages after they are stimulated with bacterial endotoxins. They activate granulocytes, which can lead to acute neutrophilic inflammation. They also induce the synthesis and release of other pro-inflammatory cytokines such as IL1, IL6 and TNFα from fibroblasts, macrophages, dendritic cells and lymphocytes.

IL8 gene: (see above).

IPA selected inflammation-related genes

TOLLIP gene (toll interacting protein): This gene encodes an ubiquitin-binding protein that interacts with several Toll-like receptor (TLR) signaling cascade components. The encoded protein regulates inflammatory signaling and is involved in interleukin-1 receptor trafficking and in the turnover of IL1R-associated kinase. [provided by RefSeq, Aug 2011] The TLR pathway is a part of the innate immune system that recognizes structurally conserved molecular patterns of microbial pathogens, leading to an inflammatory immune response.

VPREB1 gene: The protein encoded by this gene belongs to the immunoglobulin superfamily and is expressed selectively at the early stages of B cell development, namely, in proB and early preB cells. This gene encodes the iota polypeptide chain that is associated with the Ig-mu chain to form a molecular complex which is expressed on the surface of pre-B cells. The complex is thought to regulate Ig gene rearrangements in the early steps of B-cell differentiation. [provided by RefSeq, Oct 2008] It has also recently been designated CD179A.

GRN gene: Codes for the granulin protein, which regulates cell growth. However, different members of the granulin protein family may act as inhibitors, stimulators, or have dual actions on cell growth. Granulin family members are important in normal development, wound healing, and tumorigenesis.

CYP2B6 gene: This gene encodes a member of the cytochrome P450 superfamily of heme-thiolate monooxygenase enzymes, which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids.

KAT2B gene: Codes for CBP and p300 are large nuclear proteins that bind to many sequence-specific factors.

ADAR gene: This gene is upregulated upon inflammation and encodes the enzyme responsible for RNA editing by site-specific deamination of adenosines. This enzyme destabilizes double stranded RNA through conversion of adenosine to inosine. It functions to modify viral RNA genomes and may be responsible for hypermutation of certain negative-stranded viruses. It binds to ILF3/NF90 and up-regulates ILF3-mediated gene expression (GeneCard).

GAD2 gene: This gene encodes one of several forms of glutamic acid decarboxylase, identified as a major autoantigen in insulin-dependent diabetes. The enzyme encoded is responsible for catalyzing the production of gamma-aminobutyric acid from L-glutamic acid. A pathogenic role for this enzyme has been identified in the human pancreas since it has been identified as an autoantibody and an autoreactive T cell target in insulin-dependent diabetes. This gene may also play a role in the stiff man syndrome.

HLA-DRB3 gene: This gene codes for a MHC Class II molecule that plays a central role in the immune system by presenting peptides derived from extracellular proteins. Class II molecules are expressed in antigen presenting cells (APC, including B lymphocytes, dendritic cells, macrophages), which present them on the cell surface for recognition by the CD4 T-cells. Exogenous antigens that have been endocytosed by the APC are thus readily available for presentation via MHC II molecules, and for this reason this antigen presentation pathway is usually referred to as exogenous. As membrane proteins on their way to degradation in lysosomes as part of their normal turn-over are also contained in the endosomal/lysosomal compartments, exogenous antigens must compete with those derived from endogenous components. Autophagy is also a source of endogenous peptides, autophagosomes constitutively fuse with MHC class II loading compartments.

Defence Response to Bacteria

There were a number of genes that were highly upregulated (5-33 fold) in the JDRS gene expression study that are associated with defence response to invading pathogens including:

ISG15 (ubiquitin-like modifier): is a ubiquitin-like protein that becomes conjugated to many cellular proteins upon activation by interferon-alpha (IFNA; MIM 147660) and -beta (IFNB; MIM 147640) (Zhao et al., 2005 [PubMed 16009940]).[supplied by OMIM, Jul 2009] Stimulation of interferon-β-mediated innate immune mechanisms reduces the growth and replication of pathogenic rickettsiae via expression of ISG15 leading to post-translational modification of host cellular proteins during infection with this intracellular bacterium (Colonne et al 2011). (Also see USP18 below).

USP18 (ubiquitin specific peptidase 18): The protein encoded by this gene belongs to the ubiquitin-specific proteases (UBP) family of enzymes that cleave ubiquitin from ubiquitinated protein substrates. It is highly expressed in liver and thymus, and is localized to the nucleus. This protein efficiently cleaves only ISG15 (a ubiquitin-like protein) fusions, and deletion of this gene in mice results in a massive increase of ISG15 conjugates in tissues, indicating that this protein is a major ISG15-specific protease. Mice lacking this gene are also hypersensitive to interferon, suggesting a function of this protein in downregulating interferon responses, independent of its isopeptidase activity towards ISG15. [provided by RefSeq, Sep 2011] (see ISG15 above).

RSAD2 (radical S-adenosyl methionine domain containing 2) aka Viperin: Viperin is an interferon-inducible protein that inhibits the replication of a variety of viruses by apparently diverse mechanisms. In some circumstances, it also plays a role in intracellular signaling pathways. Its expression in mitochondria, revealed by infection with human cytomegalovirus, also affects cellular metabolic pathways. Expression; Viperin induction is mediated by both the classical IFN-stimulated gene induction pathway and the IFN-independent pathway. The IFN-mediated viperin gene expression is regulated by ISGF3, while IFN-independent viperin gene expression is regulated by IRF1 and IRF3 (right), which can be activated by viral factors or by the peroxisomal MAVS signaling pathway (Seo et al 2011).

IFIT2 (interferon-induced protein with tetratricopeptide repeats 2): see above.

IFIT3 (interferon-induced protein with tetratricopeptide repeats 3): associated with antiviral activity.

NOS2 (nitric oxide synthase 2, inducible): Nitric oxide is a reactive free radical which acts as a biologic mediator in several processes, including neurotransmission and antimicrobial and antitumoral activities. This gene encodes a nitric oxide synthase which is expressed in liver and is inducible by a combination of lipopolysaccharide and certain cytokines. [provided by RefSeq, Jul 2008]

OAS1 (2',5'-oligoadenylate synthetase 1, 40/46kDa): This gene encodes a member of the 2-5A synthetase family, essential proteins involved in the innate immune response to viral infection. The encoded protein is induced by interferons and uses adenosine triphosphate in 2'-specific nucleotidyl transfer reactions to synthesize 2',5'-oligoadenylates (2-5As). These molecules activate latent RNase L, which results in viral RNA degradation and the inhibition of viral replication. The three known members of this gene family are located in a cluster on chromosome 12. Mutations in this gene have been associated with host susceptibility to viral infection. Alternatively spliced transcript variants encoding different isoforms have been described. [provided by RefSeq, Jul 2008]

DEFB4 (Beta-defensin 4A, also Beta-defensin 2): Defensins form a family of microbicidal and cytotoxic peptides made by neutrophils. Members of the defensin family are highly similar in protein sequence. This gene encodes defensin, beta 4, an antibiotic peptide which is locally regulated by inflammation. [provided by RefSeq, Jul 2008]

Apoptosis and autophagy:

A key defence against intracellular parasites (eg MAP) is orderly death of the invaded cell (eg macrophage) by apoptosis or autophagy. This organised cell death allows potentially dangerous cell components to be contained and processed whilst MAP are killed. On the other hand, if the parasite is in control and can block apoptosis and autophagy this can lead to disorganised cell death and release of dangerous components, resulting in inflammation and necrosis and allowing MAP to escape the cell and invade new macrophages. The following genes associated with apoptosis and autophagy were upregulated in the JDRS study:

S100A8 and S100A9: important for resistance to invasion by pathogenic bacteria (Genecard). They up-regulates transcription of genes that are under the control of NF-kappa-B, promote phagocyte migration and infiltration of granulocytes at sites of wounding. However they are pro-inflammatory mediators in acute and chronic inflammation and up-regulate the release of IL8 that has been implicated in susceptibility to Tb and paratuberculosis (see above). Their antimicrobial and proapoptotic activity is inhibited by zinc ions.

AMBRA1 (autophagy/beclin-1 regulator 1): AMBRA1 binds preferentially the mitochondrial pool of the antiapoptotic factor BCL-2, and that this interaction is disrupted following autophagy induction.

BAG5 (BCL2-associated athanogene 5): The protein encoded by this gene is a member of the BAG1-related protein family. BAG1 is an anti-apoptotic protein that functions through interactions with a variety of cell apoptosis and growth related proteins including BCL-2, Raf-protein kinase, steroid hormone receptors, growth factor receptors and members of the heat shock protein 70 kDa family. This protein contains a BAG domain near the C-terminus, which could bind and inhibit the chaperone activity of Hsc70/Hsp70. Three transcript variants encoding two different isoforms have been found for this gene. [provided by RefSeq, Jul 2008]

BOK (BCL2-related ovarian killer): The protein encoded by this gene belongs to the BCL2 family, members of which form homo- or heterodimers, and act as anti- or proapoptotic regulators that are involved in a wide variety of cellular processes. Studies in rat show that this protein has restricted expression in reproductive tissues, interacts strongly with some antiapoptotic BCL2 proteins, not at all with proapoptotic BCL2 proteins, and induces apoptosis in transfected cells. Thus, this protein represents a proapoptotic member of the BCL2 family. [provided by RefSeq, Sep 2011]

CAPG (capping protein (actin filament), gelsolin-like): This gene encodes a member of the gelsolin/villin family of actin-regulatory proteins. The encoded protein reversibly blocks the barbed ends of F-actin filaments in a Ca2+ and phosphoinositide-regulated manner, but does not sever preformed actin filaments. By capping the barbed ends of actin filaments, the encoded protein contributes to the control of actin-based motility in non-muscle cells. Alternatively spliced transcript variants have been observed for this gene. [provided by RefSeq, Jan 2012]

CARD11 (caspase recruitment domain family, member 11): The protein encoded by this gene belongs to the membrane-associated guanylate kinase (MAGUK) family, a class of proteins that functions as molecular scaffolds for the assembly of multiprotein complexes at specialized regions of the plasma membrane. This protein is also a member of the CARD protein family, which is defined by carrying a characteristic caspase-associated recruitment domain (CARD). This protein has a domain structure

similar to that of CARD14 protein. The CARD domains of both proteins have been shown to specifically interact with BCL10, a protein known to function as a positive regulator of cell apoptosis and NF-kappaB activation. When expressed in cells, this protein activated NF-kappaB and induced the phosphorylation of BCL10. [provided by RefSeq, Jul 2008]

CASP8 (caspase 8, apoptosis-related cysteine peptidase): This gene encodes a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Caspases exist as inactive proenzymes composed of a prodomain, a large protease subunit, and a small protease subunit. Activation of caspases requires proteolytic processing at conserved internal aspartic residues to generate a heterodimeric enzyme consisting of the large and small subunits. This protein is involved in the programmed cell death induced by Fas and various apoptotic stimuli. The N-terminal FADD-like death effector domain of this protein suggests that it may interact with Fas-interacting protein FADD. This protein was detected in the insoluble fraction of the affected brain region from Huntington disease patients but not in those from normal controls, which implicated the role in neurodegenerative diseases. Many alternatively spliced transcript variants encoding different isoforms have been described, although not all variants have had their full-length sequences determined. [provided by RefSeq, Jul 2008]

CASP 9 (caspase 9, apoptosis-related cysteine peptidase): This gene encodes a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Caspases exist as inactive proenzymes which undergo proteolytic processing at conserved aspartic residues to produce two subunits, large and small, that dimerize to form the active enzyme. This protein is processed by caspase APAF1; this step is thought to be one of the earliest in the caspase activation cascade. Alternative splicing results in two transcript variants which encode different isoforms. [provided by RefSeq, Jul 2008]

CEPBE (CCAAT/enhancer binding protein (C/EBP), epsilon): The protein encoded by this gene is a bZIP transcription factor which can bind as a homodimer to certain DNA regulatory regions. It can also form heterodimers with the related protein CEBP-delta. The encoded protein may be essential for terminal differentiation and functional maturation of committed granulocyte progenitor cells. Mutations in this gene have been associated with Specific Granule Deficiency, a rare congenital disorder. Multiple variants of this gene have been described, but the full-length nature of only one has been determined. [provided by RefSeq, Jul 2008]

CIDEA (cell death-inducing DFFA-like effector a): This gene encodes the homolog of the mouse protein Cidea that has been shown to activate apoptosis. This activation of apoptosis is inhibited by the DNA fragmentation factor DFF45 but not by caspase inhibitors. Mice that lack functional Cidea have higher metabolic rates, higher lipolysis in brown adipose tissue and higher core body temperatures when subjected to cold. These mice are also resistant to diet-induced obesity and diabetes. This suggests that in mice this gene product plays a role in thermogenesis and lipolysis. Alternatively spliced transcripts have been identified. [provided by RefSeq, Aug 2010]

CIDEC (cell death-inducing DFFA-like effector c): This gene encodes a member of the cell death-inducing DNA fragmentation factor-like effector family. Members of this family play important roles in apoptosis. The encoded protein promotes lipid droplet formation in adipocytes and may mediate adipocyte apoptosis. This gene is regulated by insulin and its expression is positively correlated with

insulin sensitivity. Mutations in this gene may contribute to insulin resistant diabetes. A pseudogene of this gene is located on the short arm of chromosome 3. Alternatively spliced transcript variants that encode different isoforms have been observed for this gene. [provided by RefSeq, Dec 2010]

COX4I2 (cytochrome c oxidase subunit IV isoform 2 (lung)): Cytochrome c oxidase (COX), the terminal enzyme of the mitochondrial respiratory chain, catalyzes the electron transfer from reduced cytochrome c to oxygen. It is a heteromeric complex consisting of 3 catalytic subunits encoded by mitochondrial genes and multiple structural subunits encoded by nuclear genes. The mitochondrially-encoded subunits function in electron transfer, and the nuclear-encoded subunits may be involved in the regulation and assembly of the complex. This nuclear gene encodes isoform 2 of subunit IV. Isoform 1 of subunit IV is encoded by a different gene, however, the two genes show a similar structural organization. Subunit IV is the largest nuclear encoded subunit which plays a pivotal role in COX regulation. [provided by RefSeq, Jul 2008]

COX6A1 (cytochrome c oxidase subunit VIa polypeptide 1): Cytochrome c oxidase (COX), the terminal enzyme of the mitochondrial respiratory chain, catalyzes the electron transfer from reduced cytochrome c to oxygen. It is a heteromeric complex consisting of 3 catalytic subunits encoded by mitochondrial genes and multiple structural subunits encoded by nuclear genes. The mitochondrially-encoded subunits function in the electron transfer and the nuclear-encoded subunits may function in the regulation and assembly of the complex. This nuclear gene encodes polypeptide 1 (liver isoform) of subunit VIa, and polypeptide 1 is found in all non-muscle tissues. Polypeptide 2 (heart/muscle isoform) of subunit VIa is encoded by a different gene, and is present only in striated muscles. These two polypeptides share 66% amino acid sequence identity. It has been reported that there may be several pseudogenes on chromosomes 1, 6, 7q21, 7q31-32 and 12. However, only one pseudogene (COX6A1P) on chromosome 1p31.1 has been documented. [provided by RefSeq, Jul 2008]

COX7A2L (cytochrome c oxidase subunit VIIa polypeptide 2 like): Cytochrome c oxidase (COX), the terminal component of the mitochondrial respiratory chain, catalyzes the electron transfer from reduced cytochrome c to oxygen. This component is a heteromeric complex consisting of 3 catalytic subunits encoded by mitochondrial genes and multiple structural subunits encoded by nuclear genes. The mitochondrially-encoded subunits function in electron transfer, and the nuclear-encoded subunits may function in the regulation and assembly of the complex. This nuclear gene encodes a protein similar to polypeptides 1 and 2 of subunit VIIa in the C-terminal region, and also highly similar to the mouse Sig81 protein sequence. This gene is expressed in all tissues, and upregulated in a breast cancer cell line after estrogen treatment. It is possible that this gene represents a regulatory subunit of COX and mediates the higher level of energy production in target cells by estrogen. [provided by RefSeq, Jul 2008]

CRADD (CASP2 and RIPK1 domain containing adaptor with death domain): (CASP2 and RIPK1 domain containing adaptor with death domain) The protein encoded by this gene is a death domain (CARD/DD)-containing protein and has been shown to induce cell apoptosis. Through its CARD domain, this protein interacts with, and thus recruits, caspase 2/ICH1 to the cell death signal transduction complex that includes tumor necrosis factor receptor 1 (TNFR1A), RIPK1/RIP kinase, and numbers of other CARD domain-containing proteins. [provided by RefSeq, Jul 2008]

CTSZ (cathepsin Z): The protein encoded by this gene is a lysosomal cysteine proteinase and member of the peptidase C1 family. It exhibits both carboxy-monopeptidase and carboxy-dipeptidase activities. The encoded protein has also been known as cathepsin X and cathepsin P. This gene is expressed ubiquitously in cancer cell lines and primary tumors and, like other members of this family, may be involved in tumorigenesis. [provided by RefSeq, Oct 2008]

DAP (death-associated protein): This gene encodes a basic, proline-rich, 15-kD protein. The protein acts as a positive mediator of programmed cell death that is induced by interferon-gamma. [provided by RefSeq, Jul 2008]

GSN (gelsolin): The protein encoded by this gene binds to the "plus" ends of actin monomers and filaments to prevent monomer exchange. The encoded calcium-regulated protein functions in both assembly and disassembly of actin filaments. Defects in this gene are a cause of familial amyloidosis Finnish type (FAF). Multiple transcript variants encoding several different isoforms have been found for this gene. [provided by RefSeq, Jul 2008]

MAPK8IP1 (mitogen-activated protein kinase 8 interacting protein 1): This gene encodes a regulator of the pancreatic beta-cell function. It is highly similar to JIP-1, a mouse protein known to be a regulator of c-Jun amino-terminal kinase (Mapk8). This protein has been shown to prevent MAPK8 mediated activation of transcription factors, and to decrease IL-1 beta and MAP kinase kinase 1 (MEKK1) induced apoptosis in pancreatic beta cells. This protein also functions as a DNA-binding transactivator of the glucose transporter GLUT2. RE1-silencing transcription factor (REST) is reported to repress the expression of this gene in insulin-secreting beta cells. This gene is found to be mutated in a type 2 diabetes family, and thus is thought to be a susceptibility gene for type 2 diabetes. [provided by RefSeq, May 2011]

MT-CO2 (cytochrome c oxidase subunit II) aka COX2: Cytochrome c oxidase is the component of the respiratory chain that catalyzes the reduction of oxygen to water. Subunits 1-3 form the functional core of the enzyme complex. Subunit 2 transfers the electrons from cytochrome c via its binuclear copper A center to the bimetallic center of the catalytic subunit 1

NFKBID (NFKB nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta): May regulate the expression of IL-2, IL-6, and other cytokines through regulation on NF-kappa-B activity. Functions in the regulation of inflammatory responses. May also regulate TCR-induced negative selection of thymocytes.

OAS1(see above)

PIAS4 (protein inhibitor of activated STAT, 4): Functions as an E3-type small ubiquitin-like modifier (SUMO) ligase, stabilizing the interaction between UBE2I and the substrate, and as a SUMO-tethering factor. Plays a crucial role as a transcriptional coregulation in various cellular pathways, including the STAT pathway, the p53 pathway, the Wnt pathway and the steroid hormone signaling pathway. Involved in gene silencing. Promotes PARK7 sumoylation. In Wnt signaling, represses LEF1 and enhances TCF4 transcriptional activities through promoting their sumoylations

RNF41 (ring finger protein 41): Acts as E3 ubiquitin-protein ligase and regulates the degradation of target proteins. The encoded protein plays a role in type 1 cytokine receptor signaling by controlling the balance between JAK2-associated cytokine receptor degradation and ectodomain shedding. Negatively regulates MYD88-dependent production of proinflammatory cytokines but can promote TRIF-dependent production of type I interferon. Promotes also activation of TBK1 and IRF3. Involved in the ubiquitination of erythropoietin (EPO) and interleukin-3 (IL-3) receptors. Thus, through maintaining basal levels of cytokine receptors, FLRF is involved in the
control of hematopoietic progenitor cell differentiation into myeloerythroid lineages. Contributes to the maintenance of steady-state ERBB3 levels by mediating its growth factor-independent degradation. Involved in the degradation of the inhibitor of apoptosis BIRC6 and thus is an important regulator of cell death by promoting apoptosis. Acts also as a PARK2 modifier that accelerates its degradation, resulting in a reduction of PARK2 activity, influencing the balance of intracellular redox state.

S100A8 (S100 calcium binding protein A8): Calcium-binding protein. Has antimicrobial activity towards bacteria and fungi. Important for resistance to invasion by pathogenic bacteria. Up-regulates transcription of genes that are under the control of NF-kappa-B. Plays a role in the development of endotoxic shock in response to bacterial lipopolysaccharide (LPS) (By similarity). Promotes tubulin polymerization. Promotes phagocyte migration and infiltration of granulocytes at sites of wounding. Plays a role as pro-inflammatory mediator in acute and chronic inflammation and up-regulates the release of IL8 and cell-surface expression of ICAM1. Extracellular calprotectin binds to target cells and promotes apoptosis. Antimicrobial and proapoptotic activity is inhibited by zinc ions

S100A9 (S100 calcium binding protein A9): Calcium-binding protein. Has antimicrobial activity towards bacteria and fungi. Important for resistance to invasion by pathogenic bacteria. Up-regulates transcription of genes that are under the control of NF-kappa-B. Plays a role in the development of endotoxic shock in response to bacterial lipopolysaccharide (LPS) (By similarity). Promotes tubulin polymerization when unphosphorylated. Promotes phagocyte migration and infiltration of granulocytes at sites of wounding. Plays a role as a pro-inflammatory mediator in acute and chronic inflammation and up-regulates the release of IL8 and cell-surface expression of ICAM1. Extracellular calprotectin binds to target cells and promotes apoptosis. Antimicrobial and proapoptotic activity is inhibited by zinc ions.

THAP3 (THAP domain containing, apoptosis associated protein 3): Component of a THAP1/**THAP3**-HCFC1-OGT complex that is required for the regulation of the transcriptional
activity of RRM1.

TNFRSF17 (tumor necrosis factor receptor superfamily, member 17, death receptor 6): The protein encoded by this gene is a member of the TNF-receptor superfamily. This receptor is preferentially expressed in mature B lymphocytes, and may be important for B cell development and autoimmune response. This receptor has been shown to specifically bind to the tumor necrosis factor (ligand) superfamily, member 13b (TNFSF13B/TALL-1/BAFF), and to lead to NF-kappaB and MAPK8/JNK activation. This receptor also binds to various TRAF family members, and thus may transduce signals for cell survival and proliferation. (provided by RefSeq, Jul 2008)

TNFSF10 (tumor necrosis factor (ligand) superfamily, member 10, TRAIL): Cytokine that binds to TNFRSF10A/TRAILR1, TNFRSF10B/TRAILR2, TNFRSF10C/TRAILR3, TNFRSF10D/TRAILR4 and possibly also to TNFRSF11B/OPG. Induces apoptosis.
Its activity may be modulated by binding to the decoy receptors TNFRSF10C/TRAILR3, TNFRSF10D/TRAILR4 and TNFRSF11B/OPG that cannot induce apoptosis.

TNIK (TRAF2 and NCK interacting kinase): Serine/threonine kinase that acts as an essential activator of the Wnt signaling pathway. Recruited to promoters of Wnt target genes and required to activate their expression. May act by phosphorylating TCF4/TCF7L2. Appears to act upstream of the JUN N-terminal pathway. May play a role in the response to environmental stress. Part
of a signaling complex composed of NEDD4, RAP2A and **TNIK** which regulates neuronal dendrite extension and arborization during development. More generally, it may play a role in cytoskeletal rearrangements and regulate cell spreading.

Genome wide association studies

This subject has recently been reviewed (Purdie et al 2011) and was summarised nicely: "A genome association study is one in which a group of genetic markers that are representative of a phenotype are analysed for variation within a set of DNA samples. There are two general approaches to this type of study. The first is the candidate gene approach in which genomic variations are analysed in the context of a targeted gene or a number of genes that are believed to influence expression of complex phenotypes. The choice of genes is informed by known or suspected biological and/or physiological properties. The second approach is the genome-wide association study (GWAS) in which genetic variation across an entire genome is analysed to identify genetic association with observable traits. In the past few years the GWAS approach has been made possible in cattle and sheep by the development of whole genome arrays."

A number of genome-wide association studies on Johne's disease in livestock have been carried out in the last 10 years., including Gonda in US Holsteins (Gonda et al 2007), Settles US Holsteins (Settles et al 2009), Minozzi in Holstein cattle in Italy (Minozzi et al 2010), Pant for MAP resistance in Canadian Holsteins (Pant et al 2010), Neibergs reanalyzed dataset from Settles study (Neibergs et al 2010), Zanella identified loci associated with MAP tissue infection and tolerance to Johne's disease Holstein cattle (Zanella et al 2011a; Zanella et al 2011c) and Kirkpatrick on US Holsteins (Kirkpatrick et al 2011). These Genome wide association studies (GWAS) have been extensively reviewed (Purdie et al 2011) and the summary is presented here:

"The first GWAS seeking to identify QTL affecting the susceptibility of cattle to paratuberculosis was published in 2007 (Gonda et al 2007). The study involved Holstein half-sibling sire families that had a low relationship among the sires, with twelve sires chosen that had between them 4350 daughters. MAP status of the daughters was determined by positive results in serum ELISA and/or faecal culture and all sires were disease-free. A previous study identified low level heritability (0.102) of susceptibility to paratuberculosis in the same cohort (Gonda et al 2006). Following diagnostic testing, three families were chosen for genotyping based on high MAP infection prevalence. Two MAP negative cattle were chosen for each that tested positive, matched according to sire, herd and lactation number (Table 3).

Whole genome scans were performed using pooled DNA from cattle with similar phenotypes (MAP positive or negative). The pools were genotyped, allele frequencies estimated and then sire allele frequencies were compared between the positive and negative pools. The microsatellites (n = 291) used in this study were selected from the United States Department of Agriculture bovine linkage map and were stated to provide coverage of the bovine genome (Ihara et al 2004; Kappes et al 1997). The results identified eight chromosomal regions with putative linkage to paratuberculosis infection within at least one but not all three sire families. The author proposed that a QTL on BTA20 affects susceptibility to MAP infection.

Settles et al. (Settles et al 2009) published the first study relating to MAP susceptibility and utilising SNP arrays BovineSNP50 BeadChip). Samples from 245 Holstein dairy cattle were obtained from four geographically distinct herds in the USA, with MAP infection status assessed by faecal and tissue culture at necropsy. Results from the SNP analysis were analysed using Beadstudio software (Illumina) and a custom cluster file developed by the University of Missouri. The association of loci was tested according to the individual culture results i.e. tissue culture positive vs. negative, faecal culture positive vs. negative, tissue positive vs. faecal negative and tissue positive vs. faecal positive. The justification for these multiple methods of analysis was based on a stated lack of understanding of the pathogenesis of the disease and the suspicion that differentially identified loci may have different roles at varying stages in the progression of paratuberculosis. Although sixteen individual SNP were identified in this study, not all were statistically significant in all four diagnostic variables and none matched previously published data (Settles et al 2009). Lack of knowledge regarding gene specificity in areas associated with the SNP was proposed as a reason for lack of specific gene association, although in most cases putative genes within 1Mb were identified for each SNP. The SNP data from the above study were re-analysed in a pathway-based rather than an individual SNP approach, in an attempt to mine the data for candidate gene groups associated with susceptibility (Neibergs et al 2010). This involved pathway information from KEGG biological pathways or Gene Ontology gene sets (Table 1). Termed ‘gene-set enrichment analysis from SNP’ (GSEA-SNP), the approach identified one gene set (positive regulation of cell motion) from Gene Ontology associated with tissue culture positive samples, as well as four putative candidate genes for future study. A further GWAS published by the same group took an alternate approach, looking for genetic loci associated with tolerance rather than susceptibility to paratuberculosis (Zanella et al 2011b). This involved the same cohort of animals and genotyping data as Settles et al. (Settles et al 2009). Tolerance for this study was defined by the level of MAP faecal shedding in relation to the level of infection (identified by culture of tissues). A ‘tolerant’ animal was one that was tissue culture positive (infected) but displayed low or no faecal shedding, being distinct from resistance to MAP infection (Zanella et al 2011b). Using the same animals, different loci were identified when tolerance was defined quantitatively (as a ratio of faecal to tissue culture cfu) or qualitatively, as a case–control definition. This highlighted the complicated nature of GWAS SNP data and the importance of accurately identifying and defining the phenotype of the sample group.

Pant et al. (Pant et al 2010) utilised the BovineSNP50 array to identify genetic loci putatively associated with MAP infection. Samples were collected from Holstein cattle on six commercial farms, selected based on a previous history of MAP infection, with infection status assessed by ELISA (Table 3). The statistical analysis incorporated a novel principle component regression approach to take into account the possibility of linkage disequilibrium between SNP markers. Twenty two SNP on seven different chromosomes were identified that were significantly associated with disease. Among these, four SNP were in complete linkage. The authors assumed a single QTL in a region of linked SNP with

overlapping chromosomal regions within 1 Mb of their map position and based on this assumption calculated that the 22 SNP represent 12 QTLs. The study identified a large number of potentially significant genes including 90 associated with a QTL on BTA7 that contained four separate SNP. However, they did not find any genes matching previously published studies for MAP susceptibility.

A study conducted by Kirkpatrick et al. (2010) followed on from the work on QTL previously reported by this group (Gonda et al 2007; Kirkpatrick et al 2011). This was a larger scale genotyping project (using BovineSNP50) compared to other reported SNP GWAS for MAP, with two independent populations of Holstein cattle including over 500 MAP positive animals, tested by ELISA (Shin et al 2008) (Table 3). The design was non-typical; rather than a case–control study of animals from an 'exposed' population, they utilised genotyping information from sires used by the US dairy industry for artificial insemination as the controls without knowledge of MAP exposure. Thus the results related purely to disease susceptibility and no conclusions could be drawn regarding disease resistance. Data from the two cattle populations were analysed separately and combined with information on paternal and maternal haplotypes included where known. A strong and consistent linkage at BTA20 for one population was identified, supporting the finding made previously on this cohort of cattle based on microsatellite marker data (Gonda et al 2007; Kirkpatrick et al 2011). However, this linkage was not identified in the second population of cattle. From the 197 most significant SNP identified, a model that included 51 SNP was generated that could potentially be used as markers to predict MAP disease susceptibility. Again, there was no correlation with the findings of a previous GWAS for MAP in Holstein cattle (Settles et al 2009).

The GWAS study by Minozzi et al. (Minozzi et al 2010) was also on a very large scale (approximately 900 cattle) conducted on Holstein cattle from the Lodi province in Italy with high MAP prevalence. A similar approach to Pant et al. (Pant et al 2010) was adopted, using the BovineSNP50 and cases and controls from highly infected herds identified by ELISA alone. In order to account for the close familiar structure inherent in dairy cattle, they used a mixed model and regression that incorporated estimated relatedness. They identified several significant associations, particularly a region with several SNP markers on BTA12. An association with BTA9 was seen that correlated with an earlier study (Settles et al 2009), though other reported associations with susceptibility, such as that on BTA20, were not confirmed (Gonda et al 2007).
The authors of most studies on paratuberculosis susceptibility have been aware of the limitations in deriving accurate estimates of the size of genetic effects which are imposed by inaccurate classification of both infected animals and controls. This is common to chronic diseases and statistical modelling confirmed that the power to detect associations in paratuberculosis is generally low, although power may exist to detect larger effects and higher allele frequencies. In a frank discussion of study limitations, Kirkpatrick et al. (2010) reminded us that the results of two genome-wide scans did not correspond, due to differences in statistical power and differences in the methods of classification of the infection status of animals between the two studies.

Tables 1, 2 and 3 (below) coped from the review by Purdie et al 2011 (Purdie et al 2011).

Table 1. Genetic resources and technical platforms in sheep and cattle.

Platform	Species	Resource
Sequence	Sheep	http://www.livestockgenomics.csiro.au/sheep/vsheep.php
	Cattle	http://www.ncbi.nlm.nih.gov/projects/genome/guide/cow/
Microsatellite	Sheep	Maddox et al. (2001) (1062 loci)

Platform	Species	Resource
	Cattle	Ihara et al. (3802 microsatellites) (http://www.marc.usda.gov/genome/cattle/cattle.html)
SNP libraries	–	NCBI Entrez SNP (http://www.ncbi.nlm.nih.gov/snp)
	–	Roslin Bioinformatics resSpecies (http://www.bioinformatics.roslin.ac.uk/resspecies/)
SNP (candidate gene)	–	Custon TaqMan® SNP genotyping assay (Applied Biosystems)
	–	iPLEX® Gold MassARRAY system (Sequenom Inc.)
SNP chips	Sheep	Ovine SNP50 BeadChip (illumina) (54241 SNP)
	Cattle	BovineSNP50 BeadChip (illumina) (54609 SNP)
	Cattle	GeneChip Bovine Mapping 10K SNP (Affymetrix)
Other resources	–	KEGG biological pathways (http://www.genome.jp/kegg/pathway.html)
	Cattle	Gene Ontology (http://www.ebi.ac.uk/GOA/cow_release.html)

Table 2. Statistically significant candidate gene polymorphisms associated with MAP susceptibility.

Gene	Species	Micro-satellite	SNP	Mutation[a]	Location	Significance[b]	Reference	Year
CARD15	Cattle	–	2197T>C	Cys733Arg	LRR[c]	2.32 (1.41–3.83)[d,e]	Pinedo et al. [39] Pinedo et al. [59]	2009
	Cattle	–	1908C>T	–	3′-UTR[c]	2.043 (1.22–3.42)[f]	Ruiz-Larranaga[73]	2010
IFNγ	Cattle	–	2781G/T	Gly134Val	Exon 1	1.98 (1.11–3.51)[d,g]	Pinedo et al. [37]	2009
IL10RA	Cattle	–	984G>A	Syn [c]	Coding	2.27 (1.4–3.67)[h]	Verschoor et a [38]	2010
	Cattle	–	1098C>T	Syn	Coding	2.27 (1.4–3.67)[h]	Verschoor et a [38]	2010
	Cattle	–	1269T>C	Syn	Coding	2.27 (1.4–3.67)[h]	Verschoor et a [38]	2010
	Cattle	–	1302A>G	Syn	Coding	2.27 (1.4–3.67)[h]	Verschoor et a [38]	2010
MHC	Sheep	163/*	–	–	–	$p < 0.05$[i]	Reddacliff et al [11]	2005
SLC11A1	Sheep	162/*	–	–	Intron 1	$p < 0.05$[i]	Reddacliff et al [11]	2005
	Cattle	275/*	–	–	3′-UTR	2.01 (1.08–3.76)[e]	Pinedo et al. [37]	2009
	Cattle	279/*	–	–	3′-UTR	5.31 (1.56–17.9)[e]	Pinedo et al. [37]	2009
	Cattle	–	1067C>G	Pro356Ala	Transmembrane domain 8	1.484 (1.049–2.099)	Ruiz-Larranaga et al. [34]	2010
	Cattle	–	1157-91A>T	–	Intron 11–12	1.592 (1.095–2.314)	Ruiz-Larranaga et al. [34]	2010
TLR1	Cattle	–	G658A	Val220Met	Ectodomain	3.459	Mucha et al. [57]	2009
	Sheep	–	448A>G	Ser150Gly	Ectodomain	9.08j	Bhide et al.	2007
	Sheep	–	517G>Y	Glu173[Lys,Glu]	Coding	9.08k	Bhide et al.	2007
	Sheep	–	658A>G	Val220Met	Ectodomain	9.08j	Bhide et al.	2007
TLR2	Cattle	–	2038A>G	Ile680Val	Toll/IL-1R domain	NAl	Mucha et al. [57]	2009
	Cattle	–	1903T>C	Syn	Putative LRR	1.7 (1.2–2.8)h	Koets et al. [40]	2010
	Sheep	–	2008A>Y	Phe670Leu	Toll/IL-1R domain	4.5k	Bhide et al.	2007
	Sheep	–	2037T>C	Leu679Phe	Toll/IL-1R domain	2.01k	Bhide et al.	2007
TLR4	Cattle	–	892G>Y	Gly298[Arg,Trp]	Ectodomain	NAl	Mucha et al. [57]	2009
	Cattle	–	895G>A	Asp299Asn	Ectodomain	NAl	Mucha et al. [57]	2009
	Cattle	–	1165G>A	Gly389Ser	Ectodomain	NAl	Mucha et al. [57]	2009
	Cattle	–	1167T>C	Gly389Ser	Ectodomain	NAl	Mucha et al. [57]	2009
	Sheep	–	1066T>C	Phe356Leu	Ectodomain	1.64j	Bhide et al.	2007

a Change in the amino acid sequence of the protein, if the polymorphism is within the translated region.
b Significance presented as odds ratio (95% confidence interval) or *p* value for Chi-square/Fisher exact test.
c Abbreviations: LRR, leucine rich repeats; 3′-UTR, 3′ untranslated region; Syn, synonymous, no amino acid change.
d Odds ratio determined by univariate, ordinal (0 = homozygous, 1 = heterozygous mutant, 2 = homozygous mutant) analysis.
e Effect was also significant using multivariate analysis, considering factors such as breed and age.
f Significance for the Spanish population of Holstein-Friesian only.
g Significance was lost on multivariate analysis, considering factors such as breed and age.
h Odds ratio for the dominance effect.
i Significance of the association with severe disease.
j Odds ratio for homozygous mutant.
k Odds ratio for heterozygous mutant.
l Not applicable as 100% (14/14) of cattle with these mutations all had a disease phenotype.

Table 3. Sample numbers and classification methods.

Animal/breed	MAP pos	MAP neg	Reference	Year	Test used
Sheep					
Merino (flock A)	75	31	Reddacliff et al. [11]	2005	Faecal culture, Clinical signs, Histo[a], AGID[b], DTH[b]
Merino (flock B)	42	43	Reddacliff et al. [11]	2005	Faecal culture, Clinical signs, Histo[a], AGID[b], DTH[b]
Tsigai	82	838	Bhide et al.	2007	Clinical signs, ELISA[c] (Pourqier), PCR (blood)[d]
Cattle					
Holstein	73	158	Gonda et al.	2007	Faecal culture, ELISA (IDEXX)
Holstein/Jersey/Brahman-Angus[e]	126	305	Pinedo et al. [39]	2009	Faecal culture, ELISA, PCR[f] (Histo,PCR on subset)
Holstein/Jersey/Brahman-Angus[e]	126	305	Pinedo et al. [59]	2009	Faecal culture, ELISA, PCR[f] (Histo,PCR on subset)
Holstein/Jersey/Brahman-Angus[e]	126	305	Pinedo et al. [37]	2009	Faecal culture, ELISA, PCR[f] (Histo,PCR on subset)
Holstein	90[g]	128[g]	Settles et al. [83]	2009	Faecal culture, tissue culture (ileum and assoc. nodes)
Slovak spotted	1	9	Mucha et al. [57]	2009	Clinical signs, PCR (blood)
Slovak spotted × Holstein	0	16	Mucha et al. [57]	2009	Clinical signs
Polish red	5	59	Mucha et al. [57]	2009	Clinical signs
Holstein	27	109	Mucha et al. [57]	2009	Clinical signs
Pinzgauer	0	247	Mucha et al. [57]	2009	Clinical signs
Slovak Simmental	103	103	Mucha et al. [57]	2009	Clinical signs
Dark brown Carpathians	24	8	Mucha et al. [57]	2009	Clinical signs
Holstein-Friesian (case control)	12	12	Koets et al. [40]	2010	Faecal culture, Histo, Clinical signs
ND[h] (Confirmation study)	245	308	Koets et al. [40]	2010	Faecal culture, ELISA (Pourqier)
ND (Immune function study)	50	0	Koets et al. [40]	2010	Faecal culture, ELISA (Pourqier)
Holstein-Friesian (Spain)	129	114	Ruiz-Larranaga et al. [34]	2010	Faecal culture, PCR (faecal) ELISA
Holstein-Friesian (Netherlands)	138	177	Ruiz-Larranaga et al. [34]	2010	Faecal culture, ELISA
Holstein	90	142	Pant et al. [41]	2010	ELISA (HerdChek IDEXX) or milk ELISA
Holstein	204	242	Verschoor et al. [38]	2010	ELISA (HerdChek IDEXX) or milk ELISA
Holstein-Friesian (Spain)	127	114	Ruiz-Larranaga et al. [73]	2010	Faecal culture, PCR (faecal), ELISA
Holstein-Friesian (Netherlands)	178	226	Ruiz-Larranaga et al. [73]	2010	Faecal culture, ELISA
Holstein	90[g]	128[g]	Zanella et al. [85] Neibergs et al. [84]	2010	Faecal culture, tissue culture (ileum and assoc. nodes)
Holstein (case control study)	483	483	Minozzi et al. [88]	2010	ELISA (ID-screen)
Holstein (confirmation study)	140	137	Minozzi et al. [88]	2010	ELISA (ID-screen)
Holstein	521	1025[i]	Kirkpatrick et al. [86]	2010	Faecal culture, ELISA (Shin et al. 2008)

a Histopathology on tissues after necropsy.
b AGID: agar-gel immunodiffussion test, DTH: delayed-type hypersensitivity.
c ELISA refers to serum/plasma ELISA, with type in brackets if specified in the article.
d *IS900* PCR, with the sample type specifed in brackets.
e Holstein $n = 299$, Jersey $n = 50$ and Brahman-Angus cross $n = 82$.
f *IS900* PCR on milk, blood and faeces performed. Subset were tested by *IS900* PCR of tissues at necropsy.
g Defined by tissue culture. Alternate analyses conducted based on tissue and faecal culture results.
h ND: Not defined.
i These were used to develop a multi-marker model, otherwise the number of MAP negative controls used was not defined.

Heritability estimates of susceptibility of livestock to Johne's disease

A number of studies of heritability of paratuberculosis infection in dairy cattle have been reported from the following countries with heritability estimates in brackets:
Netherlands (0.03-0.18) based on infection status at slaughter (Koets et al 2000)
Netherlands 0.03-0.1) using milk ELISA (van Hulzen et al 2011)
Denmark (0.1) based on milk ELISA (Mortensen et al 2004)
Germany (0.1) based on serum antibody (Hinger et al 2008)
USA (0.125) based on faecal culture and serum antibody ELISA (Gonda et al 2006)
USA (0.065 to 0.095) based on milk ELISA (Attalla et al 2010)
Ireland (0.07 to 0.15) based on serology (Berry et al 2010)
They used various criteria to define infection and statistical methods, but were all around 10% and are similar to estimates reported for many other diseases (Shook 1989). In any given environment, predicted daughter prevalence of MAP infection of the highest bull was twice that of the lowest bull (Gonda et al 2006)). These studies provide evidence for the existence of important genetic variation in susceptibility to paratuberculosis.

Eight years of health records for ovine Johne's disease (OJD) from a long-term breeding trial with Romneys and Merinos were analysed by Hickey et al. to study the quantitative genetics of animal resistance/susceptibility to OJD (Hickey et al 2003). They concluded that "the heritability of lifetime incidence of OJD, considered as a binomial trait and using animal-model restricted maximum likelihood methods, was estimated as 0.07 ± 0.14 and 0.18 ± 0.11 for Romneys and Merinos respectively, giving an overall value (pooled within breeds) of 0.14 ± 0.09. Adjusting for Merino group in the relationship matrix (rather than as a fixed effect) led to a heritability for Merinos of 0.24 ± 0.09. These estimates may be biased upwards by `vertical transmission` of OJD organisms from dam to offspring. The heritability of age at death/culling had values of 0.11 ± 0.04 and 0.16 ± 0.05 for Romneys and Merinos respectively, with a pooled value of 0.13 ± 0.03. With the low heritability estimates for OJD in Romneys it is concluded that traditional breeding methods to reduce the incidence would be slow or unsuccessful; however, for Merinos, selection could begin to reduce the incidence, but ranking later generations may become difficult." (Hickey et al 2003).

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