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Combining Genome Wide Association Studies and Differential Gene Expression Data Analyses Identifies Candidate Genes Affecting Mastitis Caused by Two Different Pathogens in the Dairy Cow

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Abstract

Mastitis is a costly disease which hampers the dairy industry. Inflammation of the mammary gland is commonly caused by bacterial infection, mainly *Escherichia coli*, *Streptococcus uberis* and *Staphylococcus aureus*. As more bacteria become multi-drug resistant, one potential approach to reduce the disease incidence rate is to breed selectively for the most appropriate and potentially protective innate immune response. The genetic contribution to effective disease resistance is, however, difficult to identify due to the complex interactions that occur. In the present study two published datasets were searched for common differentially expressed genes (DEGs) with similar changes in expression in mammary tissue following intra-mammary challenge with either *E. coli* or *S. uberis*. Additionally, the results of seven published genome-wide association studies (GWAS) on different dairy cow populations were used to compile a list of SNPs associated with somatic cell count. All genes located within 2 Mbp of significant SNPs were retrieved from the Ensembl database, based on the UMD3.1 assembly. A final list of 48 candidate genes with a role in the innate immune response identified from both the DEG and GWAS studies was further analyzed using Ingenuity Pathway Analysis. The main signalling pathways highlighted in the response of the bovine mammary gland to both bacterial infections were 1) granulocyte adhesion and diapedesis, 2) ephrin receptor signalling, 3) RhoA signalling and 4) LPS/IL1 mediated inhibition of RXR function. These

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pathways comprised a network regulating the activity of leukocytes, especially neutrophils, during mammary gland inflammation. The timely and properly controlled movement of leukocytes to infection loci seems particularly important in achieving a good balance between pathogen elimination and excessive tissue damage. These results suggest that polymorphisms in key genes in these pathways such as *SELP*, *SELL*, *BCAR*1, *ACTR*3, *CXCL*2, *CXCL*6, *CXCL*8 and *FABP* may influence the ability of dairy cows to resist mastitis.

Keywords

Innate Immunity, Disease Resistance, *E. coli*, *S. Uberis*, SNP, Somatic Cell Count

1. Introduction

Mastitis in dairy cows, characterized by a high cell count in the milk, is one of the most economically serious diseases of livestock worldwide. In the UK there are around 40 cases of clinical mastitis per 100 cows. It is estimated that 70% of these are mild (being treated by the farmer), 29% are severe (requiring a visit from the veterinarian) and 1% are fatal [1]. Continuous genetic selection for milk production in modern high yielding dairy cows has been associated with an increased incidence of mastitis [2] [3]. Poor health reduces longevity and causes serious economic losses [4]. The bacteriological aetiology of mastitis has changed over recent years from primarily contagious forms (such as *Staphylococcus* (*St.*) *aureus*) to environmental pathogens (such as *Escherichia* (*E.*) *coli* and *Streptococcus* (*S.*) *uberis*) [5]. Conventional treatment requires antibiotic therapy but traditional mastitis control strategies are not fully efficient against environmental pathogens [6]. This raises major concerns over antibiotic residues in food products as well as an ever increasing rate of antimicrobial resistance [7]. Alternative options are urgently needed for efficiency, health and sustainability of the dairy industry. This need will be even more urgent in future as antibiotic usage in farm livestock becomes increasingly restricted.

Many studies have indicated that the host defence status is a key factor determining the severity of mastitis [8] [9]. The innate response offers the first line of defence in the bovine mammary gland, potentially helping the host to eliminate invading pathogens at an early stage, thus minimizing adverse effects [10]. This rapid early response is of key importance as adaptive immune responses seem to fail to induce long-lasting protection [11]. A more sustainable approach to decrease the incidence of disease and improve health in dairy cattle is thus by genetic selection, utilizing gene polymorphisms for enhanced innate immunity [12]. Such genetic improvement could potentially result in cumulative, permanent and cost-effective change. The candidate genes for genetic selection are, however, difficult to identify due to the complex interactions that must occur for effective disease resistance.

A recent UK survey showed that *S. uberis* and *E. coli* were the predominant pathogens isolated from clinical mastitis cases and *S. uberis* from subclinical cases [13]. Different bacterial species lead to different host responses [14]. Differentially expressed gene (DEGs) profile studies have provided much useful information for understanding dairy cow mastitis. These profiles have been derived from cattle challenged *in vivo* with *St. aureus* [15], *E. coli* [16] [17] and *S. uberis* [18] [19]. Additional *in vitro* studies have investigated the responses of bovine monocyte-derived macrophages to *St. aureus* [20] and mammary epithelial cells to both *St. aureus* and *E. coli* [21] [22]. These studies have revealed some important differences between Gram-negative and Gram-positive bacterial species in terms of both the time course and magnitude of the response as well as which genes are differentially expressed. Gram-negative *E. coli* infections are generally of short duration with a high bacterial count and induce a rapid and strong rise in the pro-inflammatory cytokines TNFα, IL1β and IL6 in mammary tissue via TLR4-dependent lipopolysaccharide (LPS) induced signalling. This results in a fast influx of neutrophils to inhibit bacterial growth [11]. On the other hand, Gram-positive bacteria such as *S. uberis* cause a slower and less dramatic response [23] while *St. aureus* is better able to evade the host immune response leading to a more persistent infection. In these instances, TLR signalling increases *IL*6 expression but does not up-regulate TNFα and IL1β [11]. Despite these species-specific responses, similarities still exist across bacterial species, such as the up-regulation of genes related to the innate immune response and down-regulation of genes related to fat metabolism [16] [18]. Indeed, several innate immune response pathways show evolutionary conservation independent of the infecting pathogens [24]. This increases the feasibility of finding common defence mechanisms against

mastitic infections caused by different bacterial species, which is necessary to develop a more efficient breeding strategy for dairy cows.

Allele-phenotype association studies, especially genome-wide association studies (GWAS), offer another source of valuable information for understanding genetic mechanisms underlying different dairy cow phenotypes. This provides a powerful tool to map QTL of important dairy traits onto the genome. Several GWAS using bovine SNP chips have investigated associations between mastitis incidence and somatic cell count (SCC) in different dairy cow populations [25]-[33]. SCC is generally more informative than clinical case recording for this purpose as it is recorded regularly and consistently from all cows in the herd in a more extensive and reliable manner. In general, mastitis incidence increases with increasing SCC [34] and the genetic correlation between SCC and clinical or subclinical mastitis is positive [35] [36]. There is however a U shaped distribution as cows with a very low SCC may have fewer immune cells present in their udders, making them more susceptible to infection [11]. Results from GWAS studies thus present us with a fragmented but key insight into which areas of the genome may be associated with improved resistance to mastitis.

We hypothesize that important common innate immune defence mechanisms exist in dairy cows to different sources of intra-mammary infection and that variance in the key genes associated with these mechanisms can lead to differences in disease resistance. Identifying these common and bacterial-species independent pathways is an essential step in developing an appropriate future breeding strategy. Supporting that this approach is feasible comes from a comparison of the responses of mammary epithelial cells derived from two groups of German Holstein heifers which are selected for high or low susceptibility to mastitis using marker assisted selection for a haplotype on BTA18 which is associated with SCC [22] [37]. Gene expression profiles found that the more resistant animals showed a quicker and stronger response to both *E. coli* and *St. aureus* with greater expression of the cytokines *IL1β*, *IL6*, *IL8* and *TNFα*, and the chemokines *CXCL2* and *CXCL3* and *NFKB1A*. These studies suggested that *RELB*, a transcription regulator in the NF-κB family, was the gene most likely to be responsible for the quantitative trait locus (QTL).

In the present paper, we have undertaken a systematic integrated analysis on expression and association profiling to search for such potential common defence mechanisms (innate immunity) and different genetic mechanisms (gene polymorphisms) existing in dairy cows which alter their responses to invading pathogens within the mammary gland.

2. Materials and Methods

2.1. Data and Differential Expression Analysis

Two gene expression profiles from the Gene Expression Omnibus (GEO) database (accession numbers GSE15025 and GSE15344) were used to search for common DEGs whose expression in mammary tissue samples was altered following intra-mammary challenge with either *E. coli* (using Affymetrix Bovine Genome Array, platform GPL2112) [17] or *S. uberis* (using UIUC Bostaurus 13.2 K 70-mer oligoarray (condensed), made by W.M. Keck Center, University of Illinois Urbana-Champaign, platform GPL8776) [18]. The results of these two studies were chosen for analysis because: 1) *E. coli* and *S. uberis* are representative gram negative and gram positive bacteria respectively; 2) they are both common environmental pathogens causing mastitis [5] [13]; 3) the expression profiles were generated at similar times after the start of infection (20 - 24 h), which is the stage associated with innate immunity. The microarray data were analysed using a moderated student t-test with the Benjamini-Hochberg (BH) adjustment for false discovery rate (FDR) control using GeneSpring GX12.5 software (Agilent Technologies, Santa Clara, CA), according to gene accession number and experimental group as described in the GEO database and related articles [17] [18]. Fold changes were calculated as: Infected/Control where Infected ≥ Control (up-regulation) and -Control/Infected where Infected < Control (down-regulation). P values were calculated based on a moderated t-test (tow sided) built in GeneSpring V12.5. The moderated variance was calculated as following where $S_{m1\text{-}m2}$ is the variance across the conditions, $m1$ and $m2$ are the mean expression values for gene g within groups, $df_{(m1\text{-}m2)} = n1 + n2 - 2$, S_{global} and d_{global} are the prior variance and degrees of freedom, respectively:

$$S_{mod} = \frac{S_{M1-M2} \times df_{(m1-m2)} + S_{\text{global}} \times d_{\text{global}}}{df_{(m1-m2)} + d_{\text{global}}}$$

The moderated *t* value was calculated as:

$$t_{gmod} = \frac{m1 - m2}{S_{mod}}$$

The data obtained at 20 h and 24 h respectively following intra-mammary bacterial inoculation were used for the analysis. At this time point it was considered that the treatment would have triggered an innate immune response in defence against the invading pathogens. However, as relatively few neutrophils had invaded the mammary gland by 20 h following *S. uberis* infection [18], DEG data may reflect mainly differences in gene expression of mammary epithelial cells. All the DEGs with similar expression patterns in both data sets and fold changes in response to *E. coli* infection > 1.5 or S. *uberis* infection > 2 were selected (**Table S1**). These different cut-off values were chosen due to the different microarray platforms used in the two previous experiments (*E. coli*: Affymetrix one colour/channel array; *S. uberis*: two colour/channel customised array) [17] [18]. Based on the nature of the microarray platforms, the data for the Affymetrix array were normalised using the Robust Multi-array Average (RMA) method whereas those for the two colour array were normalised with Lowess regression. Use of the different cut-off values in our study thus provided an appropriate number of genes for further analysis from each of the original data sets.

2.2. Candidate Genes Based on Genome-Wide Association Study (GWAS)

Significant SNPs identified by GWAS in seven different dairy cow populations were used to select candidate genes associated with somatic cell count (SCC) (**Table 1**). Genes located within 2 Mbp upstream and downstream of significant SNPs were retrieved from the Ensembl database with the position based on the UMD3.1 assembly at http://www.ensembl.org.The SNPs located on the same chromosome within 2 Mbp were considered as a block when looking for candidate genes. All the SNPs and adjacent genes included are listed in **Table S2**.

2.3. Data Integration of Gene Expression and GWAS

The two datasets from the differential expression and GWAS analyses were integrated. The 48 common genes which appeared on both lists represented genes with a putative role in responding to infection with organisms causing mastitis (**Figure 1** and **Table 2**).

2.4. Selection of Candidate Genes Involved in the Innate Immune Response

Annotation was initially provided by GeneSpring Technology files updated on May 2014 and gene function clustering via Ingenuity Pathway Analysis (IPA, Ingenuity Systems, Redwood City, CA. http://www.ingenuity.com). The 48 shortlisted candidate genes were further annotated using a number of databases available online, including the NetAffx™ analysis center toolbar on the Affymetrix website (http://www.affymetrix.com) and GeneCard (http://www.genecards.org). The genes were also compared with the innate immune genes database InnateDB (http://www.innatedb.com). After screening, only genes contributing to "inflammatory response" or "innate immunity response" were considered for further analysis.

Table 1. Summary of studies using GWAS to identify SNPs associated with somatic cell count in different dairy cow populations.

Population	SNP chip	No. significant SNPs	Reference
Canadian Holsteins	1536 SNP Marker Chip	11	Kolbehdari *et al.* [26]
U.S. Holsteins	BovineSNP50 Bead Chip	20	Cole *et al.* [28]
Norwegian Red cattle	Affymetrix 25K MIP Array	29	Sodeland *et al.* [29]
Netherlands Holsteins	BovineSNP50 Bead Chip	1	Wijga *et al.* [30]
Irish Holstein-Friesian	BovineSNP50 Bead Chip	5	Meredith *et al.* [31]
Nordic Holsteins	BovineSNP50 Bead Chip	21	Sahana *et al.* [32]
German Holstein	Unknown	10	Abdel-Shafy *et al.* [33]

Table 2. List of 48 candidate genes with significant changes in expression in mammary tissue in response to both *E. coli* and *S. uberis* and which were also located close to significant SNPs in gene association studies with SCC. See Materials and Methods section for details on calculations of significance. #**Bold** denotes the genes located within 1Mbp around significant SNPs associated with SCC. The remainder were within 2 Mbp.

Gene symbol#	*E. coli*		*S. uberis*		Significant SNPs
	P	Fold change	P	Fold change	
FASN	2.40E−06	−4.39	8.83E−03	−2.51	rs41257403
FABP4	3.18E−05	−2.89	2.85E−05	−2.93	rs41629827
RHPN2	3.52E−06	−2.71	5.48E−04	−2.73	rs29020544
ACSS2	4.86E−06	−2.48	7.37E−03	−2.27	rs41576572
NOV	8.64E−05	−2.10	4.87E−03	−2.36	rs41629827
ROGDI	4.29E−05	−2.08	6.60E−03	−2.32	Hapmap25382−BTC−000577
CBFA2T3	7.95E−06	−2.06	2.93E−03	−2.23	rs110754697
SORBS1	2.09E−04	−2.03	9.37E−03	−2.46	rs41650611
ALDH18A1	7.01E−07	−1.88	2.36E−03	−2.34	rs41650611
BDH2	1.03E−04	−1.82	5.96E−03	−2.40	rs41664497
FAM110A	9.80E−04	−1.62	1.08E−03	−2.30	rs41601522
HSF1	1.75E−05	−1.61	7.46E−03	−2.15	rs109421300
EMX2	1.63E−03	−1.55	5.62E−03	−2.37	rs41606777
GNAS	2.59E−04	−1.52	9.46E−03	−2.27	rs41694067
CST6	5.04E−04	−1.52	6.63E−03	−2.21	rs29027496
SRL	2.89E−03	1.51	1.05E−03	2.21	Hapmap25382−BTC−000577
SLC25A16	7.48E−03	1.52	1.50E−04	2.31	rs41655339
MRPL12	6.69E−03	1.53	8.58E−03	2.27	rs41636878
EIF4E	2.24E−05	1.54	2.11E−03	2.35	rs110927426
MAPRE1	1.44E−04	1.58	3.23E−04	2.44	rs29022774
GSPT1	7.42E−04	1.59	5.60E−03	2.42	BFGL−NGS−119848
CRNKL1	1.49E−04	1.62	4.51E−03	2.26	rs41628293
DDX27	1.15E−04	1.63	4.46E−04	2.33	rs109934030
BCAR1	2.77E−03	1.66	3.06E−05	2.54	rs29014958
TRIP10	1.78E−04	1.72	1.61E−04	2.71	rs110066189
RBM14	4.16E−05	1.76	6.57E−04	2.35	rs29027496
TARS	3.15E−04	1.78	5.80E−04	2.39	rs41578305
ZNFX1	9.71E−05	1.81	9.50E−04	2.53	rs109934030
SULF2	1.81E−05	1.89	3.73E−03	2.33	rs109934030
B4GALT5	1.21E−03	1.93	7.58E−05	2.53	rs109934030
CDC42SE2	3.07E−07	1.99	6.06E−04	2.37	rs41657989
RSL1D1	1.75E−06	2.00	1.20E−04	2.65	BFGL−NGS−119848
SLC6A9	1.11E−05	2.16	6.49E−03	2.35	rs41628293
TPM4	7.88E−06	2.22	4.11E−03	2.39	rs110213141
SELP	2.57E−03	2.24	1.03E−04	4.69	rs41579632
SPRY1	1.59E−04	2.27	8.90E−04	2.32	rs41616806
ANTXR2	2.80E−06	2.41	3.73E−03	2.35	rs41653149
ACTR3	1.26E−07	2.49	1.89E−05	2.69	BTA−47902
ZFP36L2	2.08E−07	2.75	3.29E−04	2.91	rs43673004
EHBP1L1	2.58E−07	2.76	8.50E−04	2.92	rs29027496
PIK3AP1	3.10E−06	3.03	4.64E−05	2.44	rs41650611
CTSZ	1.31E−07	3.10	1.31E−03	2.25	rs41694067
PGS1	3.22E−08	3.51	5.73E−03	2.42	rs41636878
SELL	3.70E−09	9.41	4.88E−05	2.63	rs41579632
CXCL6	3.54E−10	36.81	6.16E−04	4.01	rs41617692
EMR1	2.94E−11	63.30	8.90E−05	6.02	rs110066189
CXCL8	1.02E−09	82.99	1.33E−04	5.64	rs41617692
CXCL2	2.02E−09	94.97	4.47E−04	3.08	rs41617692

2.5. Gene Function and Pathway Analysis by IPA

The two separate and one combined gene lists were each analysed using IPA to mine the relationships via grouping DEG into known functions, pathways, and networks. Information in IPA is based primarily on human and rodent studies but is still relevant to the cow. The fold change in response to *E. coli* infection and the associated P-value data were used for the IPA analysis. All the DEGs were included without fold-change cut-off. Most genes were mapped to their corresponding gene object in the IPA Knowledge Base considering both the direct and indirect relationship. Several analyses were run including Functional Analysis, Network Generation, Canonical Pathway Analysis and Upstream Regulators Effects Analysis.Fisher's exact test with BH-FDR control at $P < 0.05$ was used to determine the biological functions and canonical pathways significantly altered by the treatment. These analyses integrate data from a variety of experimental platforms and provide insight into the most likely molecular and chemical interactions between the DEG which have been identified.

3. Results

3.1. Differential Expression of Genes Following Intra-Mammary Infection with *E. coli* or *S. uberis*

The comparison of the list of genes identified by expression microarrays at 20 - 24 h after intra-mammary infection with either *E. coli* or *S. uberis* yielded 505 common genes with a significant fold change (>1.5) in a similar direction and p-values < 0.001. Of these, 348 genes were up-regulated and 157 genes were down-regulated (**Table S1**). When these genes were imported into IPA, 504 genes were mapped based on annotation to human or mouse within the IPA Knowledge. The top ten most significant signalling pathways are listed in **Table S2**. These included IL-10 and IL-6, glucocorticoid- and ephrin-receptor signalling and activation of LXR/RXR and PPAR*α*/RXR*α*. The primary function analysis is given in **Table S3**. As expected, the most significant functions were associated with inflammatory responses, connective tissue disorders and immune cell trafficking and cell death, survival, proliferation and movement.

3.2. Candidate Genes from Mastitis Association Studies on Different Dairy Cow Populations

Based on the seven different GWAS studies listed in **Table 1** that looked for associations with SCC, a total of 94 significant SNPs were identified (**Table S4**). One SNP (BTB-00495251) could not be found in the NCBI SNP database, so 93 were used in this study. These SNPs were distributed on most chromosomes but with higher density attributed to BTA6 (n = 27), BTA13 (n = 11), BTA20 (n = 9), BTA2 (n = 7), BTA14 (n = 7) and BTA7 (n = 5). Four SNPs located in BTA6 were selected in 2 different populations (rs41588957, rs110707460, rs108988814, rs42766480).The nearest genes to these were *TMPRSS*11*F*, *DCK*, *GC* and *NPFFR*2 respectively.

   A total of 1635 protein-coding genes were located within 2 Mbp of the 93 significant SNPs (**Table S4**). These were input for IPA analysis and 1568 of them were mapped and used for further analysis. Function analysis indicated that 209 genes were related with infectious disease and 73 were involved in inflammatory responses including the genes *NFKB*1 and *CXCR*2. Primary function analysis of Molecular and Cellular Functions showed that 76 genes were involved in Cell-to-Cell Signalling and Interaction and 76 genes were related to Cellular Movement. At the Physiological System Development and Function level, 181 genes were involved in Haematological System Development and Function and 53 in Immune Cell Trafficking (**Table S5**).The top 10 canonical pathways included both agranulocyte (mononuclear leukocytes) and granulocyte adhesion and diapedesis (**Table S6**).

3.3. Common Genes from Both the Gene Expression and SCC Association Studies

When the gene lists from the two sources were compared (**Figure 1**), 48 common genes emerged. Those genes located around the significant SNPs had similar changes in expression in response to intra-mammary infection caused by either *E. coli* or *S. uberis*. Of these genes, 30 were located within 1 Mbp around the significant SNPs (**Table 2**). These included 5 genes of particular interest, *FABP*4, *SELE*, *SELP*, *CXCL*2 and *CXCL*6, since some other genes with similar function from the same families are located in close chromosome areas (**Table 3**).

   Seven genes (*EIF*4*E*, *HSF*1, *PIK*3*AP*1, *PRMT*1, *BCAR*1, *CXCL*8, *CXCL*2) were annotated as having a role in the innate immune response by the innateDB database (http://www.innatedb.com). IPA function analysis indi-

Table 3. Summary information of 11 candidate genes for disease resistance in dairy cows mediated by neutrophil diapedesis and activation.

Candidate gene[#]	Gene function by Gencard	Gene loci (bp)	Nearest significant SNP (from GWAS) & its' position (bp)	Similar nearby functional genes	Signalling pathway (from IPA)
Up-regulated					
CXCL2	Chemokine activity	BTA6: 90695494 - 90697557	rs41617692 (89505004)	*CXCL3, CXCL9, CXCL10, CXCL11, CXCL13*	Agranulocyte Adhesion and Diapedesis
CXCL6	Chemotactic for neutrophil granulocytes	BTA6: 90645964 - 90648076			
CXCL8	Attracts neutrophils, basophils and T-cells	BTA6: 90559882 - 90563647			
SELP	Mediates the interaction of activated endothelial cells or platelets with leukocytes	BTA16: 38049259 - 38080862	rs41579632 (36785401)	*SELE*	Agranulocyte Adhesion and Diapedesis
SELL	Promotes initial tethering and rolling of leukocytes in endothelia	BTA16: 38158186 - 38173226			
ACTR3	Regulates actin polymerization	BTA2: 65892592 - 65912704	BTA-47902 (65129428)		Ephrin Receptor Signalling/ Rho-GTPase signalling
BCAR1	Plays a central co-ordinating role for tyrosine kinase-based signalling related to cell adhesion	BTA18: 2613213 - 2625218	rs29014958 (1840171)		Ephrin Receptor Signalling/Integrin signalling
Down-regulated					
FABP4	Delivers long-chain fatty acids and retinoic acid to their cognate receptors in the nucleus	BTA14: 46833665 - 46838053	rs41629827 (45847610)	*FABP5, FABP9, FABP12*	LPS/IL-1 Mediated Inhibition of RXR Function
FASN	Catalyzes the formation of long-chain fatty acids	BTA19: 51384922- 51403614	rs41257403 (50647677)		LXR/RXR Signalling
GNAS	Modulator or transducer in various transmembrane signalling systems	BTA13: 58010287 - 58049012	rs110942493 (57570093)		Ephrin Receptor Signalling/Integrin signalling
SORBS1	Involves formation of actin stress fibres and focal adhesions	BTA26: 16721039 - 16919269	rs41650611 (17530482)		Ephrin Receptor Signalling/Integrin signalling

[#]Bold denotes the genes located within 1Mbp around significant SNPs associated with SCC. The remainder were within 2 Mbp.

cated that 13 genes (*CST*6, *CXCL*2, *CXCL*6, *CXCL*8, *EMR*1, *FABP*4, *HSF*1, *SELL*, *SELP*, *CTSZ*, *SORBS*, *GNAS*, *ZFP*36*L*2) were clustered into inflammatory responses. Of these 18 genes with a role in innate immunity and inflammation, 11 were mapped in Network 1 and/or the signalling pathways Granulocyte Adhesion and Diapedesis and Ephrin Receptor Signalling. This included seven genes located within 1 Mbp around the significant SNPs (**Table 3**).

When this final list of 48 genes was analysed by IPA the results remained similar to those initially detected from the 504 genes with common responses to *E. coli* and *S. uberis* (**Table 4**). Inflammatory responses were
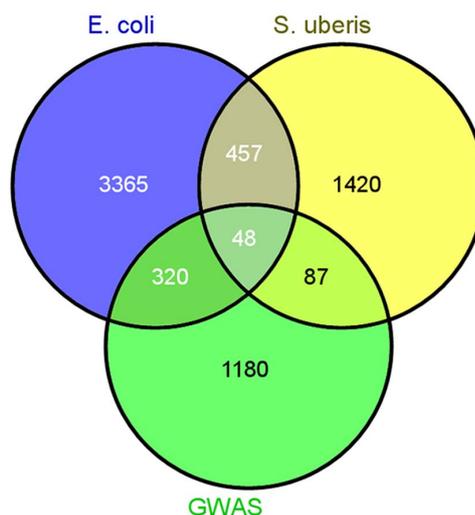
Figure 1. Venn diagram showing common genes associated with responses to mastitis in dairy cows. Candidate genes were identified using three different experimental approaches**.** Differential gene expression from mammary tissue infected with (a) *E. coli* (blue) [17] or (b) *S. uberis* (yellow) [18]. (c) A combined set of candidate genes set from seven different association studies with SCC (green) (see **Table 1** for references). A common set of 48 genes existed among the three different gene sets.

Table 4. IPA function analysis on the 48 candidate genes associated with mastitis in dairy cows in the studies of both gene expression and GWAS[*].

Name	Molecules
Diseases and Disorders	
Inflammatory Response	13
Dermatological Diseases and Conditions	9
Organismal Injury and Abnormalities	23
Gastrointestinal Disease	28
Hepatic System Disease	14
Molecular and Cellular Functions	
Cellular Movement	17
Cell-To-Cell Signalling and Interaction	13
Cell Cycle	9
Cellular Development	24
Cell Death and Survival	18
Physiological System Development and Function	
Hematological System Development and Function	16
Immune Cell Trafficking	9
Tissue Development	20
Tissue Morphology	16
Cell-mediated Immune Response	3

[*]All functions were identified using a Fisher's exact test with BH-FDR control at P < 0.05.

identified as the most significant in the Diseases and Disorders category, and Cellular movement, Cell-to-cell signalling and interaction, Cell death and survival and Cellular growth and proliferation were all important under the Molecular and Cellular Functions category. Haematological system development and function and Immune cell trafficking were the most significant under physiological system development and function. **Table 5** shows the top five canonical pathways. These included Granulocyte adhesion and diapedesis and Ephrin receptor signalling.

The physiological responses triggered by Granulocyte adhesion and diapedesis are illustrated in **Figure 2**. This shows that the neutrophil recruitment cascade is initiated by exposure of macrophages and epithelial cells

Table 5. Top canonical pathways from IPA on the 48 candidate genes associated with mastitis in dairy cows which were common in the studies of both gene expression and GWAS[*].

Top canonical pathways[+]	Changed genes found in both datasets[#]
Granulocyte Adhesion and Diapedesis	***CXCL8, SELL, SELP, CXCL2, CXCL6***
Agranulocyte Adhesion and Diapedesis	***CXCL8, SELL, SELP, CXCL2, CXCL6***
Role of IL-17A in Psoriasis	***CXCL8, CXCL6***
Ephrin Receptor Signalling	***ACTR3, BCAR1***, *GNAS, SORBS*1
Stearate Biosynthesis I (Animals)	*BDH*2, *FASN*

[+]All pathways were identified using a Fisher's exact test with BH-FDR control at P < 0.05.
[#]**Bold** denotes up-regulation and underscore denotes down-regulation of the gene.
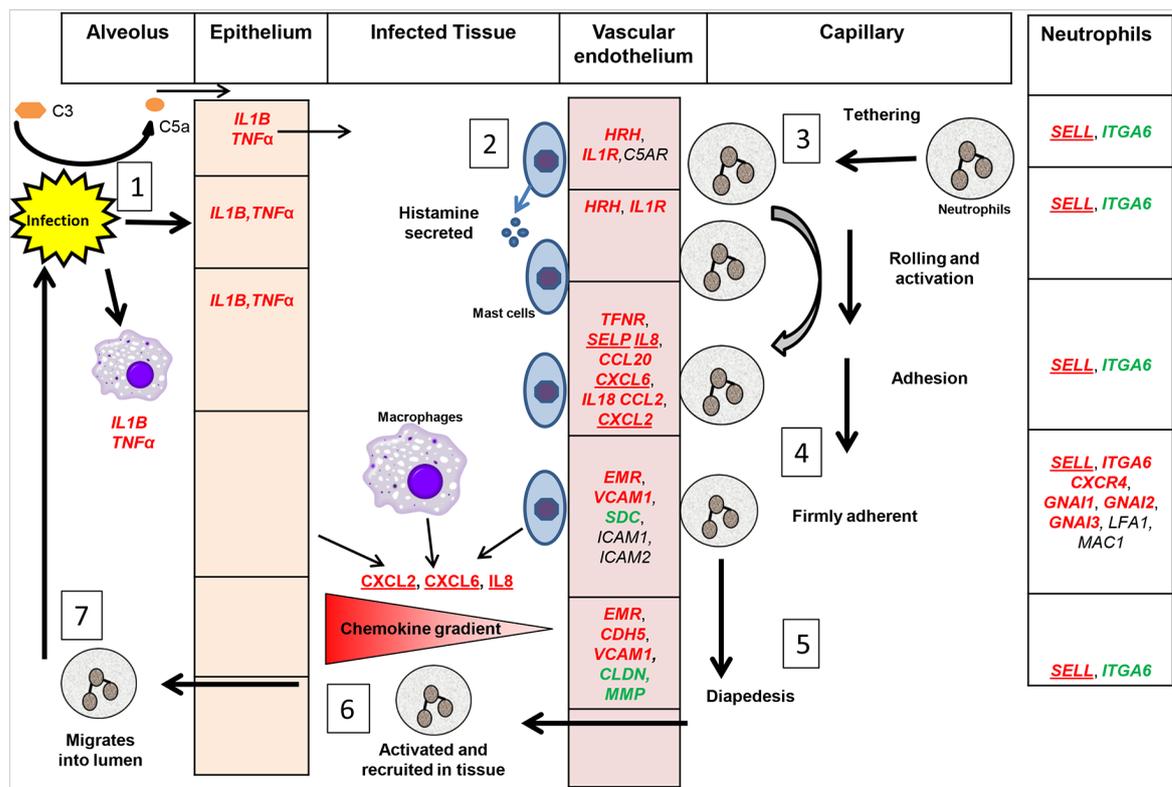
Figure 2. Summary diagram showing neutrophil adhesion and diapedesissignalling following intra-mammary infection. The 505 common DEGs following infection with either *S. uberis* or *E. coli* were mapped in IPA. Red denotes gene up-regulation and green denotes gene down-regulation. Underscore denotes genes located around significant SNPs. ((1), (2)) initiation of the neutrophil recruitment cascade by exposure of macrophages and epithelial cells to LPS or LTA inducing cytokine and complement C5a up-regulation together with histamine release from mast cells. (3) Tethering of neutrophils to the endothelium followed by rolling and activation. (4) Integrins binding to their ligands make firm adhesion between neutrophils and endothelial cells. (5) Diapedesis: neutrophils crawl to an endothelial junction then transmigrate using multiple adhesion molecules. (6) Emigrated neutrophils are recruited by moving up a new chemotactic gradient then migrating into the lumen of the mammary gland through the epithelium. (7) Activated neutrophils then play a key role in pathogen elimination.

to LPS (from Gram-negative bacteria) or lipoteichoic acid (LTA, from Gram-positive bacteria) that both induce expression of TNFα and IL1B. Complement C5a is also increased during infection and induces histamine release from mast cells [38]. TNFα, IL1β, C5a and histamine interact with their respective receptors on vascular endothelial cells and trigger their response [39] [40]. There is increased expression of the adhesion molecule P-selectin (*SELP*) following both *E. Coli* and *S. uberis* infection and during *E. coli* infection E-selectin (*SELE*) is also increased. Selectins binds their glycosylated ligands, including P-selectin glycoprotein ligand 1 (PSGL1) on the neutrophils, leading to tethering and subsequent rolling. L-selectin expression by circulating neutrophils may facilitate their secondary tethering to an already rolling neutrophil. The rolling of neutrophils facilitates their contact with the chemokine-decorated endothelium to induce activation of integrins (LFA1, MAC1). Activated integrins present higher affinity for their ligands, including immunoglobulin-like cell adhesion molecules (CAMs) [41]. Integrins binding to their ligands make firm adhesion between neutrophils and endothelial cells. Note that the crucial integrin LFA1 increases significantly in *E. coli* infection but not in *S. uberis* [16] [17]. Neutrophils then crawl to an endothelial junction by co-action of blood shear and adhesion before diapedesis (transmigration) using multiple adhesion molecules [42]. Emigrated neutrophils move up a new chemotactic gradient then migrate into the lumen of the mammary gland through the epithelium where they play a key role in pathogen elimination [43].

The physiological responses triggered by Ephrin receptor signalling are illustrated in **Figure 3**. Cytokines such as TNFα are released into the tissue where signalling via ephrin receptors causes changes in vascular endothelial cells promoting leukocytes to become adherent. This leads to cytoskeleton reorganization and neutrophil migration mainly mediated by the Rho family of small GTPases (RhoA, Cdc42, Rac) and CAS [44] [45]. Such a process would be essential in the recruitment of leucocytes into infected mammary tissue.
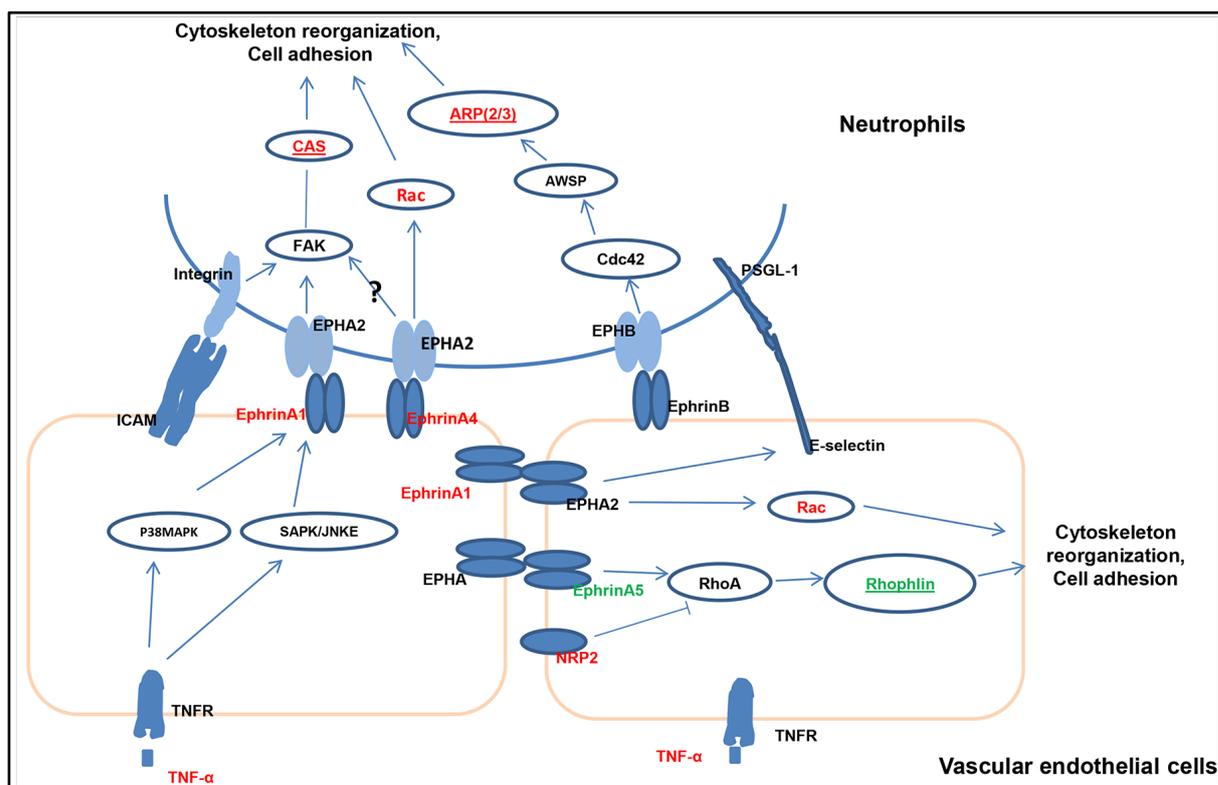
Figure 3. Summary diagram showing ephrin receptor signalling in vascular endothelial cells and neutrophils after their adhesion. The genes shown were among the common 505 DEGs following intra-mammary infection with either *S. uberis* or *E. coli*. Red denotes gene up-regulation and green denotes gene down-regulation. Underscore denotes genes located around significant SNPs. Ephrin receptor signalling changes in vascular endothelial cells in response to cytokines (e.g. TNFα) being released into the tissue and leukocytes becoming adherent to the endothelial cells. This leads to cytoskeleton reorganization and neutrophil migration mainly mediated by Rho family of small GTPases (RhoA, Cdc42, Rac) and CAS). Leukocyte migration into the mammary gland is a crucial step in the immune response.

Upstream Regulator Effect Analysis of the 48 candidate genes in IPA presented the cytokine TNFα as the one important regulator which significantly influences chemotaxis of neutrophils and their predicted activation though the transcription factor EGR1. This agrees with the finding that these two genes were both significantly up-regulated after infection by *E. coli* or *S. uberis* (**Table S1**). EGR1 in turn causes up-regulation of *CXCL*2, *CXCL*6 and *CXCL*8.

The 48 common genes which were identified from both the gene expression and GWAS studies were all contained in four main networks as revealed by IPA analysis (**Table 6**). The two most significant networks with scores > 37 were involved in: 1) Cancer, Organismal Survival, Cellular Movement and 2) Haematological System Development and Function, Humoral Immune Response, Lymphoid Tissue Structure and Development. Most of the genes annotated as innate immunity by GO terms, for example *CXCL*2, *CXCL*6, *CXCL*8, *SELL*, *SELP* and *FABP*4, were included in Network 1(**Figure S1**).

Several of the analytical approaches indicated an important role for lipid signalling, specifically long chain fatty acids (LCFA). *FASN* and *FABP*4 were the two most highly down-regulated genes from the expression studies (**Table 2**). Both these genes are involved in mammary gland lipid metabolism and are associated with SNPs identified from the GWAS studies (**Table 3**). They also both appear in Network 1 (**Table 6**). The IPA analysis of the 504 genes common to the expression studies listed LXR/RXR activation and PPARα/RXRα activation in the top 10 signalling pathways (**Table S2**). These three nuclear receptors are all known to play a role in the regulation of inflammation and in fasting mediated changes in metabolism involved in switching from utilization of glucose to LCFAs as the main source of energy [46].

4. Discussion

4.1. Experimental Approach Used

In the present study we have used the combined approach of examining previous results from both differential gene expression analysis and GWAS studies to identify the key pathways and candidate genes which potentially confer a different degree of genetic resistance to mastitis caused by bacterial pathogens in dairy cows. While this approach may be somewhat controversial, we believe that it has two main benefits. Firstly it helps to select those genes which contribute to a generalised response against all species/strains of bacteria which can cause mastitis from among the many hundreds of genes which are up- or down-regulated following a bacterial challenge. Secondly it aids in the identification of the gene(s) most likely to be causative for a QTL when examining the relevant chromosomal region. Our analysis has suggested that there are sequence variations in genes which control

Table 6. Top 4 IPA networks involving the 48 genes selected in the studies of both gene expression and GWAS.

ID	Molecules in network[#]	Score	Top diseases and functions
1	Alpha Actinin, Alpha catenin, **ANTXR2**, **BCAR1**, <u>CBFA2T3</u>, *Cg*, Collagen type IV, Collagen(s), **CXCL2**, **CXCL6**, **CXCL8**, **EIF4E**, *EMX2*, *ERK*1/2, <u>FABP4</u>, <u>FASN</u>, *Growth hormone*, <u>HSF1</u>, Hsp27, CXCL8r, LDL, Mek, N-cor, <u>NOV</u>, *Nr1h*, Pdgf (complex), *PDGF BB*, *Rock*, **SELL**, **SELP**, <u>SORBS</u>1, *Sos*, **SPRY1**, **SULF2**, Tnf (family)	40	Cancer, Organismal Survival, Cellular Movement
2	*ACKR*3, <u>ACSS2</u>, *AIMP*1, <u>ALDH18A1</u>, <u>BDH2</u>, *BRD4*, **CRNKL1**, *CSF2RB*, **DDX27**, *DNAJA3*, **EHBP1L1**, **FAM110A**, *GRB2*, *GSN*, **GSPT1**, *IRF6*, *KRT*14, *KRT*17, *LAT2*, **MAPRE1**, *MAVS*, **MRPL12**, *PGS1*, *PIK3CD*, *PTPRE*, *PTPRJ*, **RBM14**, <u>RHPN2</u>, *RSL1D1*, *S100A6*, *SH3BP2*, **TARS**, *TPM1*, **TPM4**, *UBC*	37	Hematological System Development and Function, Humoral Immune Response, Lymphoid Tissue Structure and Development
3	**ACTR3**, *ADCY*, *Akt*, *AMPK*, *Ap*1, **B4GALT5**, BCR (complex), *CD3*, **CDC42SE2**, *Creb*, **CTSZ**, *ERK*, estrogen receptor, <u>GNAS</u>, Histone h3, Ige, IgG, *IL*1, *IL*12 (complex), Immunoglobulin, Insulin, Interferon alpha, *Mapk*, *Nfat* (family), *P38 MAPK*, p85 (pik3r), *PI3K* (complex), **PIK3AP1**, *Pka*, Pro-inflammatory Cytokine, *Ras*, *TCR*, **TRIP10**, Vegf, **ZNFX1**	14	Carbohydrate Metabolism, Small Molecule Biochemistry, Cell-To-Cell Signalling and Interaction
4	*APOE*, *C5AR2*, *CARD9*, chemokine, *Ck2*, *CLEC4E*, <u>CST6</u>, dihydrotestosterone, **EMR1**, *FFAR4*, FAK, G protein, *Gsk3*, Hdac, hemoglobin, hydroquinone, *IL36G*, Il3r, *Jnk*, *KSR2*, NFkB (complex), *P2RY6*, *PELI2*, *Pik3r*, *PIK3R5*, *Pkc(s)*, RNA polymerase II, <u>ROGDI</u>, *S1PR2*, *S1PR3*, *SIGIRR*, **SLC25A16**, **SLC6A9**, **SRL**, **ZFP36L2**	13	Cell Death and Survival, Cellular Compromise, Neurological Disease

[#]Bold denotes up-regulation and <u>underscore</u> denotes down-regulation of the gene.

the movement of leukocytes, especially neutrophils, from blood to the infected mammary tissue and milk. The initial up-regulation of TNFα in response to infection induces expression of the transcription factor EGR1 [47] which plays a key role in regulating the subsequent innate immune response by in turn increasing expression of the chemokines CXCL8, CXCL2 and CXCL6. Both Gram-positive and Gram-negative bacteria can induce *EGR*1 expression in the mammary gland (**Table S1**). In the original gene expression studies analysed [17] [18], *EGR*1 expression was increased to a similar extent at 20 - 24 h following *E. coli* or *S. uberis* infection (3.05- and 2.53-fold respectively). However the downstream responses to the two pathogens were much more extreme following *E. coli* [17] with increases in *CXCL*2, *CXCL*6 and *CXCL*8 ranging from 37- to 95-fold whereas the same chemokine genes only increased by 3-5- to 6-fold following exposure to *S. uberis* [18] (**Table S1**).

Macrophages are the major cell type found in the milk of healthy bovine mammary glands while during infection an influx of neutrophils enters the infected site and plays a key role in the control of most mastitis pathogens [48]. IPA analysis showed that two pathways, Granulocyte Adhesion and Diapedesis Signalling and Ephrin Receptor Signalling seem particularly important. Granulocyte Adhesion and Diapedesis Signalling is crucial for granulocyte movement from blood vessels via the blood-udder barrier into the udder tissue [49] whereas Ephrin receptor signalling is important for mammary gland development and maintenance of function [50]. In addition, changes to lipid metabolism and/or lipid signalling through nuclear receptors are clearly also involved [11] [18]. Therefore only these three aspects of an immune response are discussed further here.

4.2. Granulocyte Adhesion and Diapedesis Signalling

Neutrophils derived from blood must experience a multistep process to reach the site of inflammation, including capture, rolling, activation and slow rolling, arrest, adhesion strengthening, intra luminal crawling followed by paracellular and transcellular migration [51]. Many of the DEGs identified in our study are known to facilitate this process (**Figure 2**). It is initiated by efficient interaction between selectins and their ligands on endothelial cells and neutrophils [52]. Increased TNFα, IL1β, C5a and histamine release could happen at a very early phase of mammary infection [53] [54]. These ligands then interact with their respective receptors TFNR, IL1R and HRH1 on the vascular endothelial cells, whose expression is also up-regulated. Such stimulation results in expression of the adhesion molecule P-selectin and VCAMs [55] [56]. P-selectin then interacts with P-selectin glycoprotein ligand 1 (PSGL-1) on the surface of neutrophils which makes them roll [57] and also primes neutrophil integrin activation for neutrophil adhesion during inflammation [58]. Integrin activation and normal function is crucial for neutrophils to develop firm adhesion after rolling. The key importance of integrinsin this process is shown by the development of the disease bovine leukocyte adhesion deficiency (BLAD) in Holstein cattle with a genetic deficiency of CD18, the β2-integrin chain [59].

L-Selectin gene expression was also significantly induced during infection. Increased L-selectin expression by circulating neutrophils could facilitate their secondary tethering to an already rolling neutrophil through attaching the PSGL-1 [60] [61]. PSGL-1 and L-selectin on the same neutrophil can interact and form a complex which is necessary to trigger integrin activation [62]. Neutrophil function was clearly associated with peripartum metritis and mastitis susceptibility [63], while glucocorticoid induced down-regulation of neutrophil L-selectin is regarded as an important reason for neutrophil dysfunction during the peripartum period [64].

The bovine selectin genes (*SELP*, *SELL*, *SELE*) are located near each other in the putative mastitis-related QTL screened by Sodeland *et al*. [29]. The QTL peak location is at 44.91cM on BTA 16. In comparison between the two gene expression studies, *SELL* was up-regulated to a greater extent than *SELP* following *E. coli* infection (9.41- v 2.24-fold) [17] whereas the reverse was true for *S. uberis* (2.63- v 4.69-fold) [18]. The fold changes in both studies were, however, of a similar order of magnitude. This putative mastitis-related region was also reported in other QTL studies [65] [66]. Furthermore, we recently found that these three genes have a high degree of polymorphism by sequencing a population of UK Holstein cows (D.C. Wathes *et al*., unpublished data). Further study will focus on determining whether these genetic variations are indeed associated with mastitis incidence rates in dairy cows.

Another important chromosomal region was located around 90 Mb on BTA6 containing the three chemokine genes *CXCL*8, *CXCL*2 and *CXCL*6. This area has been reported to show a significant relationship with mastitis in several different dairy cow populations [28] [29] [32]. All three chemokines signal via CXCR2 to activate neutrophils and subsequently promote their adhesion to the endothelium, before guiding them to move to sites of infection [67] [68]. CXCL8, released by epithelial cells, endothelial cells and macrophages is crucial for neu-

trophil activation and recruitment and has been shown to have a significant association with dairy cow mastitis [69] [70]. CXCL2, released by mast cells and macrophages, is important to control the early stage of neutrophil recruitment during tissue inflammation [71]. Indeed, a recent gene expression study revealed that *CXCL*2 was among the few significantly changed genes after experimental intra-mammary infection with *E. coli* in lactating dairy cows treated with either the antibiotic cefapirin alone, in combination with the corticosteroid prednisolone or left untreated [72]. CXCL6 released by macrophages and epithelial cells exerts neutrophil-activating and angiogenic activities [73]. Besides, it also contributes to direct antibacterial activity during localized infection [74]. Together these different studies add weight to the key importance of CXCL2 and CXCR2 in influencing the ability of the mammary gland to overcome bacterial infections successfully.

4.3. Ephrin Receptor Signalling Pathway and Its Role in Cytoskeletal Re-Organization

Ephrin receptors constitute the largest family of tyrosine kinase receptors. They may be partly responsible for the phenotypic changes to the vascular endothelium in inflammation, which allows fluid and inflamed cells to pass from the vascular space into the interstitial tissues (**Figure 3**) [75]. During infection, many cytokines are released into the mammary gland causing changed contact and signal transmission among leukocytes and vascular endothelial cells. Genes identified from our expression and association study indicated that this ephrin receptor signal can modify the cell's cytoskeleton and adhesion when leukocytes and vascular endothelial cell attract each other.

A number of genes affecting ephrin receptor signalling (*TNFα*, *IL1β*, *EphrinA*1, *SELE*, *VCAM*-1, *BCAR*1 and *ACTR*3) were all up-regulated after intra-mammary infection with *E. coli* [17]. The cascade activation of the ephrin receptor signalling process seems clear. *TNFα* is the key cytokine increased sharply by *E. coli*, which then induces EphrinA1 in endothelial cells mediated through both p38MAPK and SAPK/JNKE pathways [76]. EphA2 activation by enhanced ephrinA1 stimulates leukocyte adhesion through: 1) inducing pro-inflammatory gene expression (*SELE*, *VCAM*-1) [77]; 2) increasing vascular permeability by moving the tight and adherens junction proteins VE-cadherin and claudin-5 away from the cell surface [78]; 3) activating FAK (the protein tyrosine kinase PTK 2) and Cas (the *BCAR*1 gene product) to regulate the actin cytoskeleton and cell migration [44] [79] [80]. VCAM-1 also activates Rac1 and leads to loosening of adherens junctions [81] and induces cytoskeletal re-organization. In contrast EhrinA1 and *SELE* did not show increased expression after *S. uberis* intra-mammary infection [18] but instead only EphrinA4 was increased. One possible reason is that *S. uberis* infection induced a lower level of TNFα at an earlier time point that the 20 h used in this study [82]. Discrepant ephrin receptor signalling in response to different pathogens may cause a different clinical response: this needs to be confirmed by further study.

A significant increase in *NRP*2 and decrease in *EphrinA*5 after both types of bacterial infection [17] [18] indicated an inhibition of RhoA function [83] [84]. RhoA inactivation regulates cell migration by promoting membrane protrusion and polarity [85]. Two other important Rho-GTPases Rac1 and Cdc42 were activated during infection, maybe through EPHB receptor signalling: these in turn affect the actin polymerizing complex ARP2/3 (product of the*ACTR*3 gene) whose activation occurs through WAVE and WASP [45] [86]. ARP2/3 is crucial for cell migration by regulating the actin cytoskeleton [87] [88]. On the other hand, Rac1 and Cdc 42 activation and RhoA inactivation can also stabilize actin and help maintain endothelial cell integrity [89], which indicated that small GTPases (RhoA, Rac1, Cdc42) play an important role to maintain balance between endothelial barrier-stabilizing and increased permeability during inflammation.

4.4. Fatty Acid Metabolism and Control of Neutrophil Diapedesis

When dairy cows suffer mastitis, many genes involved in mammary gland lipid metabolism are down-regulated including *FASN*, *SCP*1, *ACC*, *LPL*, *FABP*3 and *FABP*4. Indeed, the majority of genes down-regulated by >1.5 fold following *S. uberis* infection have been associated with lipid metabolism [18]. Of these, the two genes highlighted from the GWAS studies due to their associations with SCC were *FASN* and *FABP*4 [29] [33]. The chromosome area near *FABP*4 including the tandem FABP family of genes (**Table 2**) is thus of great interest. FABP4 belongs to a family of intra-cellular lipid chaperones which bind saturated and unsaturated fatty acids, eicosanoids and other lipids with high affinity. They then transport them to specific organelles including the mitochondria for lipid oxidation, the nucleus for transcriptional regulation, theendoplasmic reticulum for membrane synthesis or lipid droplets for storage [90]. FABPs also play a role in the production of lipid mediators in-

cluding lipoxins (LXs), resolvins and protectins which have anti-inflammatory and pro-resolution properties [91] [92]. Both *FABP*3 and *FABP*4 are highly expressed in bovine mammary glands and FABP4 haplotypes have been associated with altering the proportions of saturated to unsaturated fatty acids in milk [93]. In human medicine FABP4 is attracting increasing attention as a clinical marker of metabolic disease involving insulin resistance and inflammation [90].

FASN encodes fatty acid synthase, which plays a central role in *de novo* lipogenesis in mammals. Polymorphisms in the bovine *FASN* gene also contribute to variations in milk-fat content and influence the milk fat globule composition [94]. LXRs regulate *FASN* expression through direct interaction with the FAS promoter as well as through an indirect mechanism of activation of SREBP-1c expression [95]. The milk fat globule membrane contains proteins including MFG-EGF8, which may mediate macrophage phagocytosis of apoptotic cells [96].

It is not yet clear why bacterial infection causes such a major shut-down in milk production including milk fat synthesis. One possibility would be to minimize energy loss into milk so sparing it for an effective immune response [97]. In mammary tumours, restricted access to fatty acids reduces tumour proliferation as they are essential for providing energy and for growth of the cell membrane [98]. Non-esterified fatty acids (NEFAs) and various fatty acid metabolites can also bind to and regulate the activity of PPARs, LXRs and HNF-4 [99]. A previous review indicated that FABP could combine with LTA (Leukotriene A) and stabilize it, which is helpful for the transport and transcellular biosynthesis of lipid mediators such as lipoxin A4 [100]. IPA analysis indicated that the LPS/IL1 mediated inhibition of RXR function may weaken two other metabolic signalling pathways involving LXR/RXR and PPAR/RXR. Since activation of PPAR/RXR by fatty acids can reduce inflammatory responses by many mechanisms [101], inhibition of RXR function may enhance the inflammation signal in the early stages of infection. On the other hand, direct activation of lipoxin A4 receptor (ALX) by the lipid mediator LXA4 leads to cell-type-specific signalling events that stop neutrophil migration while at the same time stimulating macrophage activation [91]. Activated macrophages enhanced phagocytosis of apoptotic neutrophils in tissue, which can weaken the IL23/IL17/G-CSF cascade for neutrophil production from bone marrow [102]. Lipid mediators may thus function as a feedback system to control an excess influx of neutrophils and so avoid tissue damage. Further work is clearly required to determine exactly how polymorphisms in *FABP*4 and *FASN* contribute to a reduction in SCC in cows.

5. Conclusion

Through integrated analysis, this study has suggested that some common innate immune signalling pathways are involved in the response of the bovine mammary gland to different types of bacterial infection. These included granulocyte adhesion and diapedesis, ephrin receptor signalling, RhoA signalling and LPS/IL1 mediated inhibition of RXR function. These signalling pathways comprised a network regulating the activity of leukocytes especially neutrophils. Many promising genes in this network are known to control a series of neutrophil functions including adhesion (*SELP*, *SELL*), diapedsis (*BCAR*1, *ACTR*3), movement (*CXCL*2, *CXCL*6, *CXCL*8) and control (*FABP*, *FASN*). Polymorphisms in these genes may thus possibly contribute to differences in disease resistance. When dairy cows suffer a mammary gland infection, the population of neutrophils in the milk and mammary gland increases significantly. Neutrophils play key roles against pathogens via diapedesis, bacterial killing and apoptosis before a final return to homeostasis. Dysfunction of each step may cause greater damage to the mammary gland. Therefore, the timely and properly controlled movement of leukocytes to infection loci seems particularly important in achieving a good balance between pathogen elimination and excessive tissue damage.

Acknowledgements

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Supporting Information

Table S1. Summary list of 505 common genes with similar differential gene expression in mammary tissue after intra-mammary infection with *E. coli* or *S. uberis*. Data are from references [17] [18].

Gene symbol	*E. coli*		*S. uberis*	
	P	Fold change	P	Fold change
TRAF3IP3	9.335E−06	−2.65	2.481E−06	−4.76
LPIN1	5.972E−03	−1.74	5.295E−05	−3.83
SLC31A2	1.933E−06	−2.58	4.977E−05	−3.38
PEG3	1.865E−04	−1.93	2.232E−04	−3.26
CD36	6.142E−10	−3.82	5.321E−05	−3.25
SLC34A2	5.118E−10	−7.01	1.370E−04	−3.21
SCD	2.713E−06	−2.25	2.693E−04	−3.11
GPAM	2.251E−07	−6.12	1.583E−06	−2.97
DNAJC12	1.111E−08	−4.66	4.942E−05	−2.95
FABP4	3.177E−05	−2.89	2.853E−05	−2.93
AAK1	2.566E−05	−1.68	1.490E−04	−2.92
TPD52L1	2.175E−04	−1.59	8.359E−04	−2.90
CLSTN1	5.645E−05	−1.91	1.222E−05	−2.87
PNPLA2	1.543E−03	−1.58	1.895E−04	−2.86
ASB11	7.854E−03	−2.04	8.502E−04	−2.83
DHRS3	4.169E−04	−1.99	5.055E−04	−2.82
CLMN	1.033E−06	−2.52	6.735E−05	−2.77
FABP3	1.644E−05	−2.98	1.164E−04	−2.75
COG2	1.221E−04	−1.51	3.650E−04	−2.75
TMEM70	1.026E−07	−2.82	9.733E−05	−2.74
ASPN	9.090E−06	−3.06	2.407E−03	−2.73
RHPN2	3.520E−06	−2.71	5.479E−04	−2.73
MUC15	2.826E−07	−2.26	3.685E−03	−2.70
CIDEA	1.352E−06	−7.65	2.019E−04	−2.69
ITGA6	8.882E−04	−1.56	1.222E−03	−2.67
SLC17A5	3.714E−03	−1.58	9.864E−04	−2.65
MOSPD1	6.276E−06	−2.61	1.756E−04	−2.63
PER1	2.920E−03	−1.72	4.145E−05	−2.62
STX8	1.417E−02	−1.57	2.552E−04	−2.62
SLC16A1	5.595E−06	−2.71	1.415E−03	−2.62
LPL	2.276E−07	−22.16	1.742E−04	−2.62
PIK3C2G	1.457E−05	−1.68	8.524E−03	−2.60
NELL2	2.098E−08	−5.15	1.865E−03	−2.60
TMCC1	9.431E−05	−1.50	3.434E−03	−2.59
PRKAR2B	9.905E−04	−1.70	1.500E−03	−2.58
MRC2	1.465E−04	−1.96	2.044E−04	−2.56
WIPI1	4.049E−03	−1.57	4.833E−05	−2.54
MYBPC1	1.715E−07	−2.81	1.187E−03	−2.53
DHCR24	2.414E−04	−1.66	1.184E−04	−2.53
ABCG2	1.573E−04	−2.01	4.777E−04	−2.52
SH3BP4	2.457E−08	−2.78	1.273E−04	−2.52
FASN	2.401E−06	−4.39	8.828E−03	−2.51
HNRPLL	3.186E−06	−2.13	1.970E−04	−2.51
ABLIM1	7.130E−04	−1.59	2.732E−04	−2.51

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*PRKAA*2	1.021E−03	−1.63	2.181E−04	−2.51
*TBC*1*D5*	1.547E−03	−1.68	2.093E−03	−2.51
VLDLR	4.323E−03	−1.95	2.147E−03	−2.50
*ITGB*5	1.213E−02	−1.60	4.420E−03	−2.50
EHF	8.660E−03	−1.50	2.412E−03	−2.50
*STK*17*A*	3.119E−06	−2.03	1.001E−03	−2.49
*ORMDL*3	8.373E−06	−2.00	2.788E−03	−2.49
*IDH*1	1.560E−06	−2.36	7.090E−04	−2.48
*PPM*1*K*	2.937E−05	−1.64	1.490E−03	−2.47
*RAPGEF*1	1.894E−06	−1.89	5.957E−04	−2.47
*SOD*1	6.628E−04	−2.21	2.019E−04	−2.47
*ESR*1	2.182E−04	−1.57	1.010E−04	−2.47
ANPEP	4.824E−07	−2.19	1.047E−04	−2.47
PTHLH	1.068E−08	−9.73	5.374E−03	−2.46
*SORBS*1	2.089E−04	−2.03	9.370E−03	−2.46
*KIAA*1370	8.537E−08	−2.48	7.122E−04	−2.46
AGRN	1.631E−04	−1.92	3.104E−04	−2.46
*BRP*44*L*	3.224E−05	−2.51	1.871E−03	−2.46
*SERTAD*4	1.191E−06	−2.99	2.089E−03	−2.45
*OSBPL*1*A*	4.526E−07	−2.58	9.569E−04	−2.45
*RECQL*5	2.916E−05	−1.73	4.821E−04	−2.45
*LRIG*1	2.639E−05	−1.67	7.424E−03	−2.45
*EFNA*5	3.052E−03	−1.52	6.255E−03	−2.45
*MMP*15	3.190E−04	−1.57	2.981E−05	−2.45
*HSD*11*B*1*L*	5.775E−04	−1.59	3.627E−03	−2.43
*COBLL*1	2.420E−05	−2.20	1.890E−03	−2.43
PRL	2.587E−04	−1.60	2.479E−03	−2.43
SLC9A3R2	1.483E−04	−1.56	4.334E−03	−2.42
*ZNF*330	3.556E−04	−1.86	9.074E−04	−2.42
*GP*2	5.675E−03	−1.77	7.272E−03	−2.42
GALM	1.447E−07	−6.95	2.480E−04	−2.42
NFIB	1.899E−03	−1.61	5.204E−03	−2.41
*IGFBP*5	1.769E−05	−2.50	1.214E−03	−2.41
*FAM*46*B*	1.360E−04	−1.61	1.457E−04	−2.41
*GGT*1	7.573E−05	−1.85	1.449E−03	−2.41
*RASSF*4	3.602E−06	−3.29	5.306E−03	−2.41
MTHFR	7.328E−04	−1.55	3.461E−03	−2.40
*SPTBN*1	3.971E−04	−1.57	2.440E−04	−2.40
*BDH*2	1.026E−04	−1.82	5.961E−03	−2.40
BAZ2B	3.091E−04	−1.62	6.260E−03	−2.40
*KLHL*21	1.051E−04	−1.52	9.846E−04	−2.39
HLF	4.224E−07	−2.97	5.134E−03	−2.39
*CD*109	2.074E−03	−1.98	4.360E−03	−2.39
*DLG*3	7.619E−04	−1.55	4.292E−03	−2.39
ANG	2.699E−06	−3.21	4.176E−03	−2.37
GTF2H5	1.433E−02	−1.56	2.333E−03	−2.37
*EMX*2	1.633E−03	−1.55	5.618E−03	−2.37
*CRISPLD*2	8.866E−07	−2.13	1.374E−03	−2.37

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GRID1	4.686E−05	−1.73	4.128E−04	−2.36
NOV	8.641E−05	−2.10	4.874E−03	−2.36
SDC2	1.457E−06	−3.23	5.085E−05	−2.36
CYB5D2	1.795E−04	−1.94	1.651E−03	−2.36
ZBTB4	5.760E−05	−1.65	8.425E−03	−2.36
TIMM8B	1.269E−02	−1.79	8.813E−05	−2.35
MIB2	3.084E−05	−1.75	3.074E−03	−2.35
GABRB3	3.532E−03	−1.51	2.399E−03	−2.35
TENC1	2.248E−05	−1.97	6.986E−04	−2.34
ALDH18A1	7.008E−07	−1.88	2.358E−03	−2.34
ENPP4	2.721E−07	−2.50	9.008E−03	−2.34
TMEM9	7.726E−04	−1.51	4.526E−03	−2.33
GUCY2D	2.993E−03	−1.52	3.144E−04	−2.33
PARD3B	3.810E−05	−1.66	4.392E−03	−2.33
ASB8	2.329E−03	−1.64	7.994E−04	−2.32
ROGDI	4.294E−05	−2.08	6.599E−03	−2.32
BEX2	4.654E−04	−1.62	9.350E−04	−2.30
FAM110A	9.800E−04	−1.62	1.075E−03	−2.30
PATZ1	2.338E−02	−1.58	9.499E−03	−2.30
TNRC6C	1.513E−04	−1.72	6.307E−04	−2.30
FXYD2	1.151E−06	−1.88	1.609E−03	−2.30
FXYD1	4.829E−03	−1.94	7.062E−03	−2.30
COL6A1	3.349E−05	−1.63	4.749E−03	−2.30
HES1	2.168E−02	−1.77	4.650E−03	−2.30
STARD13	1.808E−03	−1.61	1.833E−03	−2.30
FXYD6	6.317E−04	−1.86	3.714E−03	−2.29
GPR162	9.402E−04	−1.66	3.412E−03	−2.28
CHRNB4	1.286E−04	−1.69	6.077E−03	−2.27
CGN	4.160E−04	−1.71	4.032E−03	−2.27
GNAS	2.585E−04	−1.52	9.458E−03	−2.27
PRKCA	1.503E−05	−1.58	6.079E−03	−2.27
SHF	7.071E−04	−1.54	7.206E−04	−2.27
ACSS2	4.862E−06	−2.48	7.371E−03	−2.27
KCNMA1	4.481E−05	−1.64	3.408E−03	−2.27
CLDN16	8.120E−03	−1.58	7.603E−04	−2.26
MAGIX	5.245E−04	−1.53	5.549E−03	−2.26
CDH17	2.611E−04	−1.54	9.139E−05	−2.26
XDH	2.213E−03	−1.55	8.817E−03	−2.25
CYFIP2	4.761E−04	−1.98	3.079E−04	−2.25
ACACA	8.734E−08	−2.52	6.830E−03	−2.25
DPYD	3.994E−05	−2.32	1.369E−03	−2.25
FMOD	6.440E−03	−1.81	5.443E−03	−2.25
VGLL1	1.362E−02	−1.55	2.613E−03	−2.25
NT5E	8.932E−03	−1.87	7.080E−03	−2.24
TLN2	9.384E−06	−1.90	2.325E−03	−2.24
SLC25A21	6.866E−08	−8.53	4.211E−03	−2.24
CBFA2T3	7.948E−06	−2.06	2.934E−03	−2.23
TREX1	7.512E−03	−1.77	9.250E−03	−2.23

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TRPM4	7.061E−04	−1.70	4.594E−03	−2.22
OS9	2.494E−05	−1.61	5.856E−03	−2.22
PMM2	3.968E−05	−1.60	3.819E−03	−2.22
EFHC1	4.414E−08	−3.64	4.868E−03	−2.22
KHK	2.421E−05	−2.18	2.390E−03	−2.22
SLC6A3	2.766E−05	−1.56	8.602E−03	−2.21
TOP3B	6.568E−04	−1.56	2.844E−03	−2.21
FCHSD1	1.205E−05	−1.61	2.894E−03	−2.21
CST3	2.151E−02	−1.65	2.854E−03	−2.21
MAGED2	4.760E−04	−1.51	6.760E−03	−2.21
CST6	5.042E−04	−1.52	6.631E−03	−2.21
FGFRL1	1.201E−06	−2.27	3.069E−03	−2.20
ACO1	1.120E−04	−1.81	7.728E−03	−2.20
CUL7	3.015E−05	−1.75	3.656E−03	−2.19
CLEC3B	4.206E−04	−2.38	4.631E−03	−2.18
PON3	2.001E−02	−1.97	5.873E−04	−2.15
HSF1	1.753E−05	−1.61	7.465E−03	−2.15
RAB7A	1.967E−05	1.54	3.073E−03	2.15
ZDHHC2	6.175E−06	2.74	6.004E−03	2.16
TSPO	1.888E−06	3.40	6.837E−03	2.16
CA2	5.311E−03	2.07	6.661E−03	2.16
SMU1	4.949E−06	1.91	9.046E−03	2.17
ZNF677	1.070E−03	1.51	8.562E−03	2.17
ZFYVE26	3.997E−04	1.54	4.610E−03	2.18
CDV3	7.402E−04	1.74	6.053E−03	2.18
PLEKHO1	1.524E−08	2.99	8.724E−03	2.19
MIA3	8.766E−04	1.53	5.597E−03	2.19
RELB	3.622E−06	2.08	8.819E−03	2.19
NRP2	6.655E−04	1.83	2.940E−03	2.19
GRHPR	1.439E−05	2.11	4.284E−03	2.20
SERPINH1	2.098E−04	1.82	4.659E−03	2.20
MAD2L2	3.173E−03	1.51	1.564E−03	2.20
TLR4	1.043E−04	2.24	6.966E−03	2.21
PCNA	7.218E−05	1.53	5.080E−04	2.21
CAPZA2	8.058E−05	1.60	8.863E−03	2.21
SRL	2.891E−03	1.51	1.051E−03	2.21
BECN1	3.667E−04	1.65	6.811E−03	2.21
COX10	2.089E−05	2.03	3.098E−03	2.22
WHSC1L1	5.287E−04	1.59	6.964E−03	2.22
SRI	2.066E−07	3.16	7.708E−03	2.22
CA8	1.917E−02	1.59	8.083E−03	2.23
GNAI2	8.706E−07	2.10	7.527E−03	2.24
IFNAR2	2.013E−06	2.99	1.245E−03	2.24
GRB2	5.888E−06	1.62	3.736E−03	2.24
MIER1	7.274E−03	1.51	2.500E−03	2.24
PEX11A	1.165E−03	2.05	8.955E−03	2.24
DAZAP2	8.313E−06	1.68	4.023E−03	2.25
TWISTNB	1.188E−05	1.66	2.891E−03	2.25

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SNRPB	2.010E−03	1.56	3.837E−03	2.25
SLC3A2	1.658E−03	1.53	4.403E−03	2.25
CTSZ	1.306E−07	3.10	1.307E−03	2.25
MOCS2	1.362E−03	1.73	3.602E−03	2.25
MFAP3	3.179E−03	1.67	3.828E−03	2.26
VARS	2.488E−05	1.82	2.930E−03	2.26
SEC11A	1.321E−06	2.30	3.544E−03	2.26
HSPD1	9.354E−06	2.64	9.238E−03	2.26
FAS	8.817E−04	2.14	1.836E−03	2.26
CRNKL1	1.488E−04	1.62	4.508E−03	2.26
FARSB	1.613E−05	1.66	4.306E−03	2.26
ARF4	1.041E−08	3.69	4.812E−03	2.27
DPH5	1.484E−05	2.09	8.743E−03	2.27
MRPL12	6.693E−03	1.53	8.579E−03	2.27
CDK5RAP3	1.873E−04	1.50	1.936E−03	2.27
VASP	1.438E−07	2.28	8.285E−03	2.27
PHF11	1.431E−07	2.22	3.035E−03	2.28
HRH1	8.081E−03	2.65	9.276E−03	2.28
S100A11	5.258E−05	2.05	6.033E−03	2.28
KLF2	2.040E−04	1.82	3.530E−03	2.28
KCTD10	7.294E−05	1.68	1.770E−03	2.28
NRAS	1.649E−04	1.69	7.555E−03	2.28
PRDX1	7.507E−03	1.51	1.301E−03	2.29
VCP	5.817E−05	1.79	1.632E−03	2.29
ZFAND3	5.642E−05	1.87	1.562E−03	2.29
SKIL	1.472E−03	1.79	1.215E−03	2.29
ZSWIM6	4.863E−03	1.50	8.707E−03	2.30
CDH5	1.068E−04	1.94	2.921E−03	2.30
FSTL1	1.039E−02	1.56	5.136E−04	2.30
RNF8	1.296E−03	1.68	5.728E−03	2.30
ZMAT2	2.119E−04	1.54	8.800E−04	2.31
RBMS1	2.548E−06	2.01	6.531E−03	2.31
SNRPG	1.034E−02	1.55	4.420E−03	2.31
PSMD14	3.426E−07	2.28	6.370E−03	2.31
SLC25A16	7.479E−03	1.52	1.501E−04	2.31
KPNA3	1.404E−04	1.75	6.568E−04	2.32
PDIA4	3.298E−03	1.53	5.377E−03	2.32
SPRY1	1.586E−04	2.27	8.901E−04	2.32
PSMB2	2.769E−03	1.75	3.871E−03	2.32
ATP13A3	2.832E−03	1.56	6.209E−04	2.33
ENPP2	2.497E−02	1.56	6.808E−03	2.33
RNPS1	7.156E−06	1.83	2.321E−03	2.33
DDX27	1.147E−04	1.63	4.456E−04	2.33
PRMT1	1.998E−03	1.52	7.071E−03	2.33
DBI	7.271E−04	1.67	4.683E−04	2.33
FKBP3	1.764E−03	1.53	4.260E−03	2.33
SULF2	1.815E−05	1.89	3.735E−03	2.33
PSMB8	4.842E−05	2.47	7.027E−03	2.34

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RARS	2.396E−06	1.98	2.038E−03	2.34
*GOT*2	6.968E−03	1.60	1.631E−03	2.34
*ME*1	9.092E−03	1.68	6.852E−03	2.34
*RBBP*5	1.153E−02	1.59	1.321E−03	2.34
*CXCR*4	6.181E−06	4.51	4.472E−03	2.34
RAP2B	2.668E−05	1.77	2.376E−03	2.34
*USP*12	1.689E−06	2.09	9.603E−04	2.35
*DLG*5	1.250E−03	1.82	2.159E−04	2.35
CDKN1A	7.194E−05	5.18	4.882E−03	2.35
*FBXL*5	9.989E−06	1.97	2.323E−04	2.35
*SLC6A*9	1.106E−05	2.16	6.493E−03	2.35
*TAP*1	1.197E−06	2.59	1.247E−03	2.35
*IFIT*5	8.429E−06	2.24	2.574E−05	2.35
*RBM*22	4.014E−06	1.84	2.014E−03	2.35
*SOAT*1	5.305E−06	2.54	1.341E−03	2.35
EIF4E	2.237E−05	1.54	2.108E−03	2.35
*FAM103A*1	1.258E−06	2.09	9.365E−03	2.35
*ANTXR*2	2.796E−06	2.41	3.734E−03	2.35
*RBM*14	4.155E−05	1.76	6.572E−04	2.35
CTSB	2.620E−07	2.80	3.950E−03	2.36
*ME*2	1.431E−07	4.44	3.003E−03	2.36
*COTL*1	1.094E−07	3.71	1.556E−04	2.36
*HSP90AB*1	1.240E−07	3.63	6.663E−04	2.36
*PSME*3	4.748E−04	1.81	2.798E−03	2.36
*PSMB*4	1.045E−04	1.76	1.372E−04	2.36
*DEGS*1	1.503E−05	1.62	9.150E−04	2.36
*ZC3HAV*1	1.779E−07	3.50	1.930E−03	2.37
*IER*3	1.614E−04	2.86	5.038E−03	2.37
*TACSTD*2	7.811E−09	10.88	1.470E−03	2.37
SYNCRIP	3.443E−03	1.67	1.880E−03	2.37
*ARPC*5	2.118E−05	1.76	5.749E−04	2.37
*CDC42SE*2	3.071E−07	1.99	6.058E−04	2.37
*DNAJC*3	3.095E−05	1.81	4.416E−03	2.37
*PES*1	1.935E−05	1.54	4.557E−03	2.38
*FBXW*2	3.293E−06	2.08	4.617E−05	2.38
*DPH*2	3.106E−05	1.77	1.713E−03	2.38
*HSPA*4	4.265E−05	1.85	2.294E−03	2.38
PICALM	3.741E−04	1.82	2.153E−03	2.38
AMMECR1L	3.784E−04	1.83	2.581E−03	2.38
*CAP*1	8.545E−06	2.04	1.670E−03	2.39
*CAPZA*1	3.759E−04	1.53	1.233E−03	2.39
*TPM*4	7.875E−06	2.22	4.105E−03	2.39
TARS	3.153E−04	1.78	5.801E−04	2.39
IL4R	2.288E−05	2.33	3.517E−03	2.40
*PTGES*3	3.662E−06	1.78	1.549E−04	2.40
YWHAG	3.233E−06	2.07	1.174E−03	2.40
*BTG*1	6.532E−08	2.70	8.086E−03	2.40
*GLUD*1	2.363E−04	1.76	1.060E−03	2.40

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EBNA1BP2	1.131E−06	2.38	2.017E−03	2.40
XPO6	1.902E−07	2.11	1.043E−03	2.41
JUNB	4.860E−08	5.21	1.237E−04	2.41
DNTTIP2	2.495E−03	1.57	4.512E−03	2.41
STX6	9.094E−04	1.79	3.418E−03	2.41
YWHAZ	7.522E−04	1.64	3.060E−03	2.42
PARP14	2.667E−08	3.12	4.282E−03	2.42
CEBPB	1.907E−07	2.98	4.832E−04	2.42
RNF149	1.105E−06	2.10	3.332E−04	2.42
RPS6KA3	4.263E−04	2.89	1.932E−03	2.42
APEX1	4.864E−04	1.53	3.798E−03	2.42
IL10RB	5.428E−10	3.62	1.152E−03	2.42
EAF1	2.498E−05	2.25	4.242E−03	2.42
SLC39A6	9.024E−04	1.79	4.306E−03	2.42
GSPT1	7.421E−04	1.59	5.596E−03	2.42
PKIG	5.177E−03	1.66	2.364E−03	2.42
IQGAP1	2.597E−06	1.84	3.464E−03	2.42
ZNF281	6.625E−07	2.97	2.326E−03	2.42
PGS1	3.224E−08	3.51	5.726E−03	2.42
PSMA1	1.157E−06	2.25	1.928E−03	2.42
TNFRSF1A	1.580E−07	2.36	4.401E−05	2.42
PARP9	1.020E−09	4.19	1.971E−03	2.43
LYPLA1	8.530E−07	2.47	1.166E−03	2.43
TRIM37	5.108E−06	2.07	6.723E−03	2.44
PIK3AP1	3.097E−06	3.03	4.637E−05	2.44
SPG21	7.031E−07	2.39	1.963E−03	2.44
TSFM	2.131E−04	1.71	6.485E−03	2.44
MAPRE1	1.438E−04	1.58	3.229E−04	2.44
CD44	1.031E−09	6.88	2.314E−03	2.44
ABL1	5.007E−03	1.72	5.638E−04	2.45
PSMA2	2.987E−05	2.06	1.131E−04	2.45
LEO1	7.417E−04	1.60	9.995E−04	2.45
PSMA5	7.350E−04	1.61	1.087E−03	2.45
PREP	1.248E−05	1.96	3.760E−04	2.45
TGFBI	1.135E−07	5.74	2.525E−03	2.46
CD164	3.472E−04	1.90	8.728E−05	2.46
UNC93A	1.108E−03	1.65	1.030E−04	2.46
GPBP1	1.232E−03	1.55	4.448E−03	2.46
GNAI3	3.707E−06	2.24	1.525E−04	2.46
RAB5C	1.378E−05	1.60	2.148E−04	2.46
MAPK6	9.939E−04	1.64	9.782E−04	2.47
CDO1	3.795E−06	2.69	2.869E−05	2.47
ASNSD1	3.104E−05	1.98	4.060E−03	2.47
NFKB2	4.783E−07	5.02	3.360E−03	2.47
GAPDH	7.628E−11	3.76	7.598E−06	2.47
TXN	1.079E−05	2.98	1.756E−03	2.47
RAC1	2.337E−09	2.70	7.588E−05	2.48
CCNL1	9.457E−05	2.07	4.973E−03	2.48

Continued

*ADAM*19	4.315E−08	3.43	2.584E−03	2.48
*SH3RF*1	5.814E−05	2.48	2.488E−03	2.49
*LCP*1	4.898E−10	7.99	1.107E−03	2.49
*TNIP*1	8.206E−08	4.14	2.118E−03	2.50
*SKAP*2	7.477E−08	2.77	8.721E−04	2.50
*GNAI*1	1.874E−02	1.70	1.962E−03	2.50
*LETM*1	4.601E−04	1.77	1.214E−04	2.50
*EIF*2*AK*2	7.598E−06	2.81	9.093E−04	2.50
PYGL	2.604E−04	2.37	4.496E−04	2.50
*SPCS*3	1.244E−04	1.81	3.180E−03	2.50
*APBB*1*IP*	2.283E−07	3.71	7.966E−04	2.50
*MPEG*1	2.572E−04	2.12	3.364E−04	2.51
*BCL*6	7.592E−04	1.78	7.308E−04	2.51
TAPBP	1.496E−06	1.98	1.392E−04	2.51
MSN	1.163E−06	2.19	3.891E−03	2.51
*GFPT*2	9.933E−05	2.93	1.238E−03	2.52
*B4GALT*5	1.215E−03	1.93	7.578E−05	2.53
*KIAA*0020	3.397E−06	1.80	7.147E−03	2.53
*CNN*2	2.288E−06	2.87	6.993E−04	2.53
*ZNFX*1	9.714E−05	1.81	9.497E−04	2.53
*PLAC*8	5.976E−05	3.96	2.621E−04	2.53
*NINJ*1	1.400E−05	2.55	3.734E−04	2.53
*EGR*1	3.004E−05	3.05	3.466E−03	2.53
*BCAR*1	2.774E−03	1.66	3.061E−05	2.54
*CD*53	1.136E−08	7.50	4.943E−05	2.54
*EIF*2*S*1	1.423E−05	1.90	6.079E−04	2.54
*XPNPEP*1	3.125E−04	1.51	9.498E−05	2.55
*ETF*1	2.442E−05	2.31	2.361E−03	2.55
*CHSY*1	1.503E−03	1.87	2.960E−05	2.56
*CCT*2	4.623E−07	2.35	3.598E−04	2.56
RAN	1.566E−06	1.93	8.518E−05	2.56
MTDH	2.474E−06	2.25	6.644E−05	2.58
*FTH*1	3.253E−08	2.25	4.562E−03	2.58
YARS	2.835E−03	1.70	3.182E−04	2.59
CALCRL	6.151E−03	2.35	3.593E−04	2.59
*CD*14	5.675E−05	2.31	1.200E−03	2.59
*LAP*3	3.410E−07	2.39	1.532E−04	2.59
*DGAT*2	7.155E−06	2.49	4.294E−03	2.59
*RIPK*2	1.435E−04	1.77	1.281E−03	2.59
*SLC*39*A*1	1.189E−05	1.86	3.585E−04	2.59
ELL	1.914E−04	1.63	6.155E−05	2.59
*ATP*1*B*3	1.059E−05	1.99	3.262E−04	2.59
*HK*3	3.267E−06	2.56	4.901E−03	2.60
*PFN*1	2.672E−05	2.00	6.449E−05	2.60
*PSME*2	7.783E−05	2.25	3.356E−04	2.60
*TNFRSF*6*B*	4.761E−08	16.81	3.746E−03	2.61
*SLC*11*A*2	1.086E−04	2.94	2.950E−04	2.61
*PTGS*2	8.825E−04	2.98	3.098E−03	2.62

Continued

*ZNF*259	1.571E−08	4.09	3.524E−05	2.62
CTTN	2.266E−05	1.82	1.846E−05	2.62
*BASP*1	1.025E−09	4.61	9.302E−04	2.62
*PDIA*3	7.205E−04	1.54	2.188E−04	2.62
*TRIM*25	2.671E−03	2.10	5.664E−05	2.63
*KLF*10	3.607E−05	2.74	6.115E−05	2.63
SELL	3.699E−09	9.41	4.881E−05	2.63
*REXO*2	9.668E−03	1.51	1.273E−05	2.64
*CLEC*2D	2.334E−05	2.51	4.824E−04	2.64
*PLSCR*1	3.972E−04	1.94	1.051E−03	2.65
*RSL*1D1	1.747E−06	2.00	1.205E−04	2.65
*SLCO*4A1	2.121E−03	2.05	7.528E−05	2.65
*TSR*1	1.721E−06	2.04	2.136E−03	2.65
*MTHFD*2	4.432E−05	3.47	4.381E−04	2.66
FBL	3.675E−04	1.75	1.039E−03	2.66
*DDX*21	7.622E−04	1.91	7.045E−04	2.66
*CCT*3	8.468E−08	2.61	9.208E−04	2.67
UGDH	1.774E−08	5.49	7.768E−04	2.68
AATF	1.800E−08	2.60	1.649E−03	2.68
*PPP*1R3C	2.221E−02	1.73	5.321E−04	2.68
*TUBB*6	9.833E−06	2.01	7.009E−04	2.69
*RAC*2	3.791E−07	3.47	9.361E−05	2.69
*ACTR*3	1.263E−07	2.49	1.890E−05	2.69
*HIF*1A	5.376E−03	1.56	1.820E−04	2.70
*TRIP*10	1.781E−04	1.72	1.609E−04	2.71
*TGIF*1	2.745E−09	4.70	2.080E−03	2.72
*IL*18	4.383E−06	3.95	4.682E−03	2.73
WTAP	6.029E−07	3.51	1.929E−03	2.73
*TLR*2	3.800E−06	5.08	7.792E−04	2.73
*IL*13RA1	6.900E−08	4.46	1.827E−04	2.74
*RAB*8B	4.100E−06	2.65	8.962E−05	2.74
*DNAJB*1	1.964E−04	1.87	7.543E−05	2.74
WARS	2.972E−07	3.75	1.061E−04	2.75
*NPM*1	1.562E−06	1.91	6.562E−07	2.75
*MKNK*1	5.162E−07	4.35	8.468E−04	2.76
*PPP*2R3C	1.962E−07	2.84	9.361E−04	2.76
*DENND*2D	2.413E−05	2.11	3.423E−04	2.76
CREM	4.351E−07	2.86	7.710E−05	2.77
CFLAR	4.988E−05	3.68	5.098E−05	2.78
*IL*1RAP	7.593E−08	12.26	1.450E−03	2.79
*SMAD*3	3.316E−04	1.88	8.698E−05	2.79
CTSH	1.083E−08	4.37	4.274E−04	2.79
PTPRC	2.453E−07	3.01	7.043E−04	2.80
*EFHD*2	2.703E−04	2.29	5.093E−05	2.80
MYC	1.141E−05	2.77	1.663E−03	2.80
*SPINT*1	7.591E−07	1.98	5.885E−04	2.81
*TNFAIP*6	1.118E−05	5.40	2.746E−04	2.82
*TREM*1	2.224E−12	18.55	1.376E−03	2.82

Continued

PDK4	2.015E−05	4.29	2.598E−03	2.82
CD48	2.297E−07	7.34	3.839E−03	2.83
GNG5	7.598E−05	2.21	1.247E−04	2.83
IER5	6.385E−09	4.52	9.829E−06	2.83
PLAU	6.120E−07	3.15	7.076E−04	2.84
ARG2	5.740E−07	9.36	8.823E−04	2.85
NCL	1.458E−05	1.67	2.094E−05	2.85
SOCS3	1.981E−07	10.69	7.538E−05	2.85
CKAP4	7.588E−09	2.68	4.360E−04	2.85
FGL2	6.026E−07	3.84	2.331E−03	2.87
NOLC1	1.100E−04	1.73	3.343E−05	2.87
MFAP1	2.666E−05	1.94	3.058E−05	2.87
STAT3	3.920E−06	3.56	7.192E−04	2.87
ITGAV	8.828E−05	3.04	7.350E−04	2.89
MAP3K8	4.641E−08	6.06	2.059E−03	2.90
ZFP36L2	2.083E−07	2.75	3.291E−04	2.91
OLR1	5.814E−08	10.47	8.629E−04	2.91
EHBP1L1	2.585E−07	2.76	8.502E−04	2.92
F13A1	6.370E−05	3.35	8.009E−04	2.92
VCAM1	1.063E−05	3.70	3.358E−05	2.96
PDPN	3.439E−07	5.14	8.817E−04	3.01
FAM49B	1.473E−09	4.03	1.273E−03	3.01
HPRT1	4.758E−09	2.83	2.945E−05	3.01
IL10RA	7.765E−06	2.80	2.024E−04	3.06
CXCL2	2.015E−09	94.97	4.469E−04	3.08
IL18BP	4.330E−13	19.99	2.502E−05	3.09
CALR	1.175E−03	1.51	5.533E−05	3.10
PHC2	3.577E−05	1.94	4.503E−03	3.12
HSPA8	2.282E−04	1.97	1.316E−04	3.15
MARCKS	1.100E−06	3.38	2.273E−04	3.16
PSTPIP2	1.473E−09	9.30	8.168E−04	3.20
SPI1	1.665E−09	8.31	2.253E−03	3.22
PTX3	6.138E−05	14.95	2.374E−05	3.26
CCL20	1.492E−03	4.25	3.011E−04	3.28
GNL3	6.264E−04	1.51	8.200E−04	3.32
IL1R2	1.442E−07	21.40	4.001E−04	3.39
THBS1	1.473E−02	1.92	3.659E−04	3.44
CASP4	8.023E−09	5.76	2.035E−04	3.48
LDHA	2.002E−10	7.36	2.267E−04	3.51
ERRFI1	1.041E−06	3.51	9.545E−06	3.53
CD9	1.353E−06	1.87	3.570E−06	3.66
ZFP36	1.841E−07	3.88	1.258E−04	3.66
MXD1	1.177E−07	4.43	2.527E−05	3.76
FOS	1.259E−07	7.79	1.195E−05	3.78
CEBPD	1.642E−06	5.41	9.992E−05	3.93
BCL3	1.501E−05	2.11	1.060E−04	3.94
CXCL6	3.538E−10	36.81	6.165E−04	4.01
CD69	6.630E−06	7.08	1.879E−03	4.04

Continued

SDCBP	1.953E−06	2.14	3.513E−05	4.06
LTF	3.092E−04	2.16	2.757E−07	4.08
*CD*55	7.343E−11	18.46	2.532E−04	4.20
*EIF4A*1	7.736E−08	2.11	7.705E−05	4.25
*BTG*2	1.734E−03	1.93	8.071E−04	4.37
NFKBIA	2.947E−05	3.40	5.504E−05	4.48
*IL*1*RN*	4.433E−09	30.47	7.596E−05	4.54
*TGM*3	1.511E−10	34.32	1.288E−03	4.65
*CCR*1	1.818E−12	22.15	1.624E−04	4.67
*TIMP*1	5.574E−07	9.62	4.653E−05	4.68
SELP	2.566E−03	2.24	1.030E−04	4.69
*FSCN*1	1.983E−04	1.59	1.680E−05	4.69
RRAD	1.179E−04	2.54	1.228E−04	4.74
*IL*6	1.157E−03	7.04	5.230E−04	4.89
*CXCL*8	1.015E−09	82.99	1.332E−04	5.64
PLAUR	5.943E−04	2.24	5.168E−05	5.71
*S*100*A*2	4.128E−10	12.75	1.518E−04	5.86
*EMR*1	2.942E−11	63.30	8.899E−05	6.02
CTSC	2.926E−11	8.53	1.140E−05	6.51
SRGN	8.525E−11	10.29	8.856E−06	6.70
*LOC*508666	6.325E−04	4.39	2.602E−05	6.84
LYZ	2.947E−07	9.10	8.344E−05	6.93
*SLC*2*A*3	7.758E−06	4.59	1.488E−04	7.64
*BCL*2*A*1	1.008E−08	9.25	9.684E−05	8.12
*S*100*A*12	1.315E−12	121.83	4.831E−04	9.02
*BIRC*3	1.024E−06	9.27	2.839E−05	9.61
*MARCKSL*1	4.891E−06	2.49	1.610E−06	9.83
*S*100*A*9	1.288E−15	262.26	3.313E−05	12.22
*CCL*2	1.797E−06	11.15	2.960E−05	20.21

Table S2. Top 10 signalling pathways from IPA among the 505[#] common DEGs altered by experimental infections with both *E. coli* and *S. uberis* (all with P < 10E−6).

Canonical pathways	Molecules	Ratio
IL-10 Signalling	16	2.05E−01
Granulocyte Adhesion and Diapedesis	24	1.32E−01
LXR/RXR Activation	19	1.37E−01
IL-6 Signalling	18	1.45E−01
Glucocorticoid Receptor Signalling	26	8.7E−02
Ephrin Receptor Signalling	21	1E−01
Agranulocyte Adhesion and Diapedesis	21	1.09E−01
PPARα/RXRα Activation	20	1E−01
Hepatic Fibrosis/Hepatic Stellate Cell Activation	17	1.1E−01
Leukocyte Extravasation Signalling	20	9.52E−02

[#]504 Genes with known IPA-eligible human and rodent orthologs were considered.

Table S3. Primary function analysis with IPA for the 505[#] common DEGs altered by both experimental infections with both *E. coli* and *S. uberis* (all with P < 1E−7).

Name	Molecules
Diseases and Disorders	
Inflammatory Response	164
Connective Tissue Disorders	112
Molecular and Cellular Functions	
Cell Death and Survival	246
Cellular Growth and Proliferation	249
Cellular Movement	172
Cellular Function and Maintenance	187
Cellular Development	223
Physiological System Development and Function	
Immune Cell Trafficking	110
Organismal Survival	182
Tissue Morphology	155
Hematological System Development and Function	165
Tissue Development	149

[#]504 Genes with known IPA-eligible human and rodent orthologs were considered.

Table S4. Summary list of 93 significant SNPs associated with somatic cell count in seven different populations of dairy cows and 1635 genes located 2 Mbp around them[#].

SNP[+]	BTA	Symbols of nearby genes located within 2 Mbp
rs41637122	1	*APP*, *ATP5J*, *GABPA*, *JAM*2, *MRPL*39
BTA-47902	2	*ACTR*3, *GPR*39, *LYPD*1, *MGAT*5, *NCKAP*5, *SLC35F*5
Hap-map29674-BTA-160310/rs110139290	2	*ARHGAP*15, *KYNU*, *LRP*1*B*
rs110491622		*ABCB*11, *B3GALT*1, *BBS*5, *CCDC*173, *CERS*6, *DHRS*9, *FASTKD*1, *G6PC*2, *KBTBD*10, *KLHL*23, *LRP*2, *METTL*5, *MYO3B*, *NOSTRIN*, *PHOSPHO*2, *PPIG*, *SPC*25, *SSB*, *STK*39, *UBR*3, *XIRP*2
rs29025784	2	*TNS*1, *AAMP*, *ABCB*6, *NKZF*1, *ARPC*2, *ASIC*4, *ATG9A*, *BCS1L*, *C2H2ORF*24, *C2ORF*17, *C2orf*62, *CCDC*108, *CDK5R*2, *CHPF*, *CRYBA*2, *CTDSP*1, *CXCR*2, *CXCR*2, *CYP27A*1, *DES*, *DNAJB*2, *DNPEP*, *EPHA*4, *FEV*, *GLB1L*, *GMPPA*, *GPBAR*1, *IHH*, *INHA*, *MGC*157327, *NHEJ*1, *OBSL*1, *PLCD*4, *PNKD*, *PRKAG*3, *PTPRN*, *RECS*1, *RESP*18, *RNF*25, *RQCD*1, *SLC11A*1, *SLC23A*3, *SLC4A*3, *SPEG*, *STK11IP*, *STK*16, *STK*36, *TMEM*198, *TTLL*4, *TUBA1D*, *TUBA4A*, *USP*37, *VIL*1, *WNT10A*, *WNT*6, *ZFAND2B*, *ZNF*142
rs41622740/rs41622742	2	*ACADL*, *CPS*1, *CRYGF*, *ERBB*4, *KANSL1L*, *LANCL*1, *MAP-*2, *MYL*1, *PTH2R*, *RPE*, *UNC*80
rs29020694	4	*ING*3, *CPED*1, *WNT*16, *FAM3C*, *PTPRZ*1, *AASS*, *FEZF*1, *CADPS*2, *TAS2R*16, *SLC13A*1, *IQUB*, *NDUFA*5, *ASB*15, *LMOD*2, *WASL*, *HYAL*4, *GPR*37, *POT*1
rs41590209	4	*AASS*, *ASB*15, *CADPS*2, *CPED*1, *FAM3C*, *FEZF*1, *GPR*37, *HYAL*4, , *NG*3, *IQUB*, *LMOD*2, *NDUFA*5, *POT*1, *PTPRZ*1, *SLC13A*1, *TAS2R*16, *WASL*, *WNT*16, *AHCYL*2, *ARF*5, *ATP6V1F*, *CALU*, *CCDC*136, *CEP*41, *COPG*2, *CPA*1, *CPA*4, *CPA*5, *FAM71F*1, *FAM71F*2, *FLNC*, *FSCN*3, *GCC*1, *HIG*2, *IMPDH*1, *IRF*5, *KLHDC*10, *LRRC*4, *MEST*, *MGC*127766, *MKLN*1, *NRF*1, *OB*, *OPN1SW*, *PAX*4, *PODXL*, *PRRT*4, *RBM*28, *SMO*, *SND*1, *STRIP*2, *TMEM*209, *TNPO*3, *TSGA*13, *TSPAN*33, *UBE2H*, *ZC3HC*1, *ZNF*800
Hap-map55237-rs29010308	5	*ALX*1, *LRRIQ*1, *MGAT4C*, *NTS*, *RASSF*9, *SLC6A*15, *TSPAN*19

Continued

rs41257360	5	*APOLD*1, *BCL2L*14, *BOTA-T2R*10*B*, *CDKN*1*B*, *CREBL*2, *CSDA*, *DDX*47, *DUSP*16, *EMP*1, *ETV*6, *GPR*19, *GPRC5A*, *GPRC5D*, *GRIN2B*, *GSG*1, *HEBP*1, *KIAA*1467, *KLRA*1, *KLRC*1, *KLRJ*1, *LOH*12*CR*1, *LRP*6, *MAGOHB*, *MANSC*1, *NKG2A*, *STYK*1, *T2R*10*C*, *T2R*12, *T2R65A*, *TAS2R*10, *TAS2R*42, *TAS2R*46
rs29017739/rs29001782/ rs41588957/Hapmap6022 4-rs29001782/rs1103209 75/rs110289032/rs416109 91/rs109427807/rs811503 88/rs110707460/rs110527 224/rs43338539/rs108988 814/rs42766480/rs427664 80/ rs41617692/rs41655332/ rs41655339/rs41617947/r s41653149	6	*LPHN*3, *ADAMTS*3, *AFM*, *AFP*, *ALB*, *AMBN*, *AMTN*, *ANKRD*17, *ANTXR*2, *ANXA*3, *ART*3, *BMP2K*, *BTC*, *CABS*1, *CCDC*158, *CCNG*2, *CCNI*, *CDKL*2, *CENPC*1, *CNOT6L*, *COX*18, *CSN*1*S*1, *CSN*1*S*2, *CSN*2, *CSN*3, *CXCL*10, *CXCL*11, *CXCL*13, *CXCL*2, *CXCL*3, *CXCL*6, *CXCL*9, *DCK*, *DKFZP564O*0823, *DYNLL*1, *ENAM*, *EPGN*, *EPHA*5, *EREG*, *FAM47E-STBD*1, *FRAS*1, *G3BP*2, *GC*, *GK*2, *GNRHR*, *GRO*1, *GRSF*1, *HSTN*, *IGJ*, *CXCL*8, *MGC*152010, *MOB*1*B*, *MRPL*1, *MTHFD2L*, *NAA*11, *NAAA*, *NPFFR*2, *NUP*54, *ODAM*, *PAQR*3, *PF*4, *PPBP*, *PPEF*2, *RASSF*6, *RCHY*1, *RPL23A*, *RUFY*3, *SCARB*2, *SDAD*1, *SHROOM*3, *SLC25A*16, *SLC4A*4, *SOWAHB*, *STAP*1, *STATH*, *SULT*1*B*1, *SULT*1*E*1, *TECRL*, *THAP*6, *TMPRSS*11*A*, *TMPRSS*11*D*, *TMPRSS*11*F*, *UBA*6, *UGT2A*3, *USO*1, *UTP*3, *YTHDC*1
rs41664497/ rs110927426	6	*ADH*4, *ADH*5, *ADH*6, *ADH*7, *BANK*1, *BDH*2, *C4orf*17, *CENPE*, *CISD*2, *DAPP*1, *DDIT4L*, *DNAJB*14, *EIF4E*, *EMCN*, *H2AFZ*, *LAMTOR*3, *MANBA*, *METAP*1, *MGC*157237, *MTTP*, *NFKB*1, *NHEDC*1, *PPP3CA*, *RAP*1*GDS*1, *SLC39A*8, *SLC9B*2, *TRMT*10*A*, *TSPAN*5
rs29013992/rs109746386	6	*LPHN*3
rs110066189	7	*ACER*1, *ACP*5, *ACTL*9, *ADAMTS*10, *ALKBH*7, *ANGPTL*4, *ANGPTL*6, *AP*1*M*2, *ATG4D*, *C19orf*38, *C19orf*45, *C19orf*59, *C19orf*80, *C*3, *C7H19orf*52, *C7H19orf*66, *CAMSAP*3, *CARM*1, *CCDC*151, *CCDC*159, *CCL*25, *CD*209, *CD*320, *CD*70, *CDC*37, *CDKN2D*, *CERS*4, *CLEC4G*, *CLPP*, *CNN*1, *COL5A*3, *CTXN*1, *DENND*1*C*, *DNM*2, *DNMT*1, *DOCK*6, *ECSIT*, *EIF3G*, *ELAVL*1, *ELAVL*3, *ELOF*1, *EMR*1, *EPOR*, *EVI5L*, *FBN*3, *FBXL*12, *FCER*2, *FDX*1*L*, *GPR*108, *GTF2F*1, *ICAM*1, *ICAM*3, *ICAM*4, *ICAM*5, *ILF*3, *IR-A*, *KANK*2, *KANK*3, *KEAP*1, *KHSRP*, *KRI*1, *LDLR*, *LRRC8E*, *MAP2K*7, 02-*Mar*, *MCOLN*1, *MLLT*1, *MRPL*4, *MYO*1*F*, *NDUFA*7, *OLFM*2, *OR2Z*1, *P2RY*11, *PCP*2, *PDE4A*, *PEX*11*G*, *PIN*1, *PNPLA*6, *PPAN-P2RY*11, *PRAM*1, *PRKCSH*, *PSPN*, *QTRT*1, *RAB*11*B*, *RAB3D*, *RAVER*1, *RDH*8, *RETN*, *RGL*3, *RPS*28, *S*1*PR*2, *S*1*PR*5, *SH2D3A*, *SLC25A*23, *SLC25A*41, *SLC44A*2, *SNAPC*2, *SPC*24, *STXBP*2, *SWSAP*1, *TIMM*44, *TMED*1, *TMEM*205, *TNFSF*14, *TNFSF*9, *TRAPPC*5, *TRIP*10, *TSPAN*16, *TUBB4A*, *TYK*2, *VAV*1, *XAB*2, *YIPF*2, *ZGLP*1, *ZNF*266, *ZNF*358, *ZNF*414, *ZNF*558, *ZNF*653
rs110213141	7	*AKAP*8, *AKAP8L*, *AP*1*M*1, *BRD*4, *C19orf*44, *CALR*3, *CASP*14, *CCDC*105, *CHERP*, *CIB*3, *CYP4F*2, *CYP4F*22, *CYP4F*3, *EPHX*3, *EPS15L*1, *FAM32A*, *ILVBL*, *NOTCH*3, *OR*10*H*1, *OR7C*2, *PGLYRP*2, *RAB8A*, *RASAL*3, *SLC*1*A*6, *SYDE*1, *TPM*4, *WIZ*
rs41657989/ Hap-map50931-BTA-31235/ rs41596179	7	*7SK*, *ACSL*6, *ADAMTS*19, *ALDH7A*1, *C5orf*63, *C7H5orf*48, *CDC42SE*2, *CHSY*3, *CSF*2, *CTXN*3, *FBN*2, *FNIP*1, *GRAMD*3, *HINT*1, *IL*3, *ISOC*1, *KIAA*1024*L*, *LMNB*1, *LYRM*7, *MEGF*10, *P4HA*2, *PDLIM*4, *PHAX*, *PRRC*1,, *SLC*12*A*2, *SLC27A*6, *ZNF*608
rs41665468	9	*ADGB*, *GRM*1, *KATNA*1, *LATS*1, *LRP*11, *MGC*133692, *NUP*43, *PCMT*1, *PPIL*4, *PPP*1*R*14*C*, *RAB*32, *RAET*1*G*, *RYK*, *SAMD*5, *SASH*1, *STXBP*5, *TAB*2, *ULBP*3, *ZC3H*12*D*
rs41654612	10	*ADCK*1, *AHSA*1, *ALKBH*1, *ANGEL*1, *C14orf*166*B*, *DIO*2, *GSTZ*1, *IRF2BPL*, *ISM*2, *KIAA*1737, *NGB*, *NOXRED*1, *NRXN*3, *POMT*2, *SAMD*15, *SLIRP*, *SNW*1, *SPTLC*2, *TMED*8, *TMEM63C*, *VASH*1, *VIPAS*39, *ZDHHC*22
rs43619106	10	*AQR*, *C10H15ORF*41, *DPH*6, *FAM98B*, *MEIS*2, *RASGRP*1, *SPRED*1, *TMCO5A*, *ZNF*770
rs43673004	11	*ABCG*5, *ABCG*8, *ATP6V*1*E*2, *CAMKMT*, *CRIPT*, *DYNC2LI*1, *EPAS*1, *LRPPRC*, *MCFD*2, *PIGF*, *PLEKHH*2, *PPM*1*B*, *PREPL*, *PRKCE*, *RHOQ*, *SIX*2, *SIX*3, *SLC3A*1, *SOCS*5, *SRBD*1, *THADA*, *TMEM*247, *TTC7A*, *ZFP36L*2
rs109934030/ rs41634110/ rs109441194	13	*ARFGEF*2, *ATP9A*, *B4GALT*5, *BCAS*4, *CSE*1*L*, *DDX*27, *DPM*1, *FAM65C*, *KCNB*1, *KCNG*1, *MOCS*3, *NCOA*3, *NFATC*2, *PARD6B*, *PREX*1, *PTGIS*, *PTPN*1, *RNF*114, *SALL*4, *SLC9A*8, *SNAI*1, *SPATA*2, *STAU*1, *SULF*2, *TMEM*189, *TSHZ*2, *UBE2V*1, *ZFP*64, *ZNF*217, *ZNFX*1
rs111004585	13	*BTBD*3, *C20ORF*13, *C20ORF*82, *JAG*1, *MKKS*, *SLX4IP*, *SNAP*25, *SPTLC*3, *SRY*
rs41628293	13	*ARMC*4, *BAMBI*, *BANF*2, *BFSP*1, *C13H20ORF*12, *C20orf*26, *CRNKL*1, *CSRP2BP*, *DSTN*, *DTD*1, *MGME*1, *MKX*, *MPP*7, *NAA*20, *OVOL*2, *PCSK*2, *POLR3F*, *RAB*18, *RBBP*9, *RIN*2, *RPL*5, *RRBP*1, *SCP2D*1, *SEC23B*, *SLC24A*3, *SLC6A*9, *SNX*5, *WAC*, *ZNF*133

Continued

rs41694067/ rs110942493/rs41601522 /rs29022774/rs29022775 / rs41576572	13	*ACSS*2,, *ACTL*10, *AHCY*, *ANKRD*60, *APCDD*1*L*, *ASIP*, *ASXL*1, *ATP5E*, *AURKA*, *BASE*, *BCL2L*1, *BMP*7, *BORIS*, *BPIFA*1, *BPIFA*2*B*, *BPIFB*1, *BPIFB*2, *BPIFB*3, *BPIFB*4, *BPIFB*6, *BSP30C*, *BSP30D*, *C*13*H*20*ORF*108, *C*13*H*20*ORF*71, *C*13*H*20*ORF*85, *C*20*orf*112, *C*20*orf*144, *C*20*ORF*18, *C*20*orf*96, *CASS*4, *CBFA2T*2, *CCM2L*, *CDH*26, *CDH*4, *CDK5RAP*1, *CEP*250, *CHMP4B*, *COMMD*7, *COX4I2*, *CSNK2A*1, *CSTF*1, *CTSZ*, *DEFB*119, *DEFB*122, *DEFB*122*A*, *DEFB*123, *DEFB*124, *DEFB*129, *DNMT3B*, *DUSP*15, *DYNLRB*1, *E2F*1, *EDEM*2, *EDN*3, *EIF2S*2, *EIF*6, *FAM*110*A*, *FAM*209*B*, *FAM*217*B*, *FAM*83*C*, *FKBP*1*A*, *FOXS*1, *GCNT*7, *GDF*5, *GGT*7, *GNAS*, *GSS*, *HCK*, *HM*13, *ID*1, *ITCH*,*KIF3B*, *MAP*1*LC3A*, *MAPRE*1, *MMP*24, *MYH*7*B*, *MYLK*2, *NCOA*6, *NECAB*3, *NELFCD*, *NPEPL*1, *NRSN*2, *NSFL*1*C*, *PCK*1, *PDRG*1, *PHACTR*3, *PIGU*, *PLAGL*2, *PMEPA*1, *POFUT*1, *PPP*1*R3D*, *PROCR*, *PSMF*1, *PXMP*4, *RAB22A*, *RAD21L*1, *RAE*1, *RALY*, *RBM*38, *REM*1, *RSPO*4, *RTFDC*1, *SDCBP*2, *SIRPB*2, *SIRPD*, *SLC52A*3, *SLMO*2, *SNPH*, *SNTA*1, *SPAG4L*, *SPO*11, *SRXN*1, *SS*18*L*1, *STX*16, *SYCP*2, *TAF*4, *TBC*1*D*20, *TCF*15, *TFAP2C*, *TM9SF*4, *TMEM*74*B*, *TP53INP*2, *TPX*2, *TRIB*3, *TRPC4AP*, *TTLL*9, *TUBB*1, *UQCC*, *VAPB*,, *XKR*7, *ZBP*1, *ZCCHC*3, *ZNF*341, *ZNF*831
rs109146371/ rs109421300/ rs109350371	14	*ADCK*5, *ARC*, *ARHGAP*39, *BAI*1, *C*14*H*8*orf*82, *C8orf*33, *CCDC*166, *COMMD*5, *CPSF*1, *CYC*1, *CYHR*1, *DENND*3, *DGAT*1, *EEF*1*D*, *EXOSC*4, *FAM*83*H*, *FBXL*6, *FOXH*1, *GML*, *GPAA*1, *GPR*172*B*, *GPR*20, *GPT*,*GRINA* ,*GSDMD* ,*HSF*1 ,*KIAA*1875 ,*KIFC*2 , *LRRC*14, *LRRC*24, *LY6D*, *LY6E*, *LY6H*, *LY6K*, *LYNX*1, *LYPD*2, *MAF*1, *MAFA*, *MAPK*15, *MFSD*3, *MROH*1, *MROH*6, *NAPRT*1, *NRBP*2, *OPLAH*, *PARP*10, *PLEC*, *PPP*1*R*16*A*, *PSCA*, *PTK*2, *PTP4A*3, *PUF*60, *PYCRL*, *RECQL*4, *RHPN*1, *RPL*8, *SCRIB*, *SCRT*1, *SHARPIN*, *SLC39A*4, *SLC45A*4, *SLURP*1, *SPATC*1, *THEM*6, *TIGD*5, *TMEM*249, *TMEM*60, *TONSL*, *TSNARE*1, *TSTA*3 , *VPS*28, *ZC3H*3, *ZNF*16, *ZNF*34, *ZNF*623, *ZNF*7
rs29012803	14	*ANXA*13, *ATAD*2, *DERL*1, *FAM*83*A*, *FAM*91*A*1, *FBXO*32, *FER*1*L*6, *H3F3C*, *HAS*2, *KLHL*38, *PRKDC*, *SPIDR*, *TBC*1*D*31, *UBE2V*2, *WDYHV*1, *ZHX*1, *ZHX*2
rs41629827/ rs41630587/ rs41620570	14	*AARD*, *COLEC*10, *CSMD*3, *EIF3H*, *EXT*1, *FABP*12, *FABP*4, *FABP*5, *FABP*9, *HEY*1, *IL*7, *MAL*2, *MED*30, *MRPS*28, *NOV*, *PAG*1, *PKIA*, *PMP*2, *RAD*21, *SAMD*12, *STMN*2, *TNFRSF*11*B*, *TPD*52, *TRPS*1, *UTP*23, *ZBTB*10,*ZC2HC*1*A* ,*ZNF*704
rs110034639	16	*ESRRG*, *GPATCH*2, *LYPLAL*1, *RRP*15, *TGFB*2, *USH2A*
rs41579632	16	*ATP*1*B*1, *BLZF*1, *C*1*orf*112, *CCDC*181, *CEP*170, *CHML*, *DPT*, *EXO*1, *F*5, *FH*, *KIFAP*3, *KMO*, *MAP*1*LC3C*, *METTL*11*B*, *METTL*18, *NME*7, *PLD*5, *RGS*7, *SCYL*3, *SELE*, *SELL*, *SELP*, *SLC*19*A*2, *WDR*64, *XCL*1
rs41616806	17	*ANKRD*50, *BBS*12, *CETN*4, *FAT*4, *FGF*2, *NUDT*6, *SPATA*5, *SPRY*1
rs110754697	18	*ACSF*3, *ANKRD*11, *APRT*, *BANP*, *C*16*orf*95, *C*18*H*16*orf*87, *CA5A*, *CBFA2T*3, *CDH*15, *CDK*10, *CDT*1, *CHMP*1*A*, *CPNE*7, *CTU*2, *CYBA*, *DBNDD*1, *DEF*8, *DNAJA*2, *DPEP*1, *FANCA*, *FBXO*31, *FOXC*2, *FOXF*1, *FOXL*1, *GALNS*, *GAS*8, *GPT*2, *IL*17*C*, *IRF*8, *ITFG*1, *JPH*3, *KLHDC*4, *LAT*, *MAP*1*LC3B*, *MC*1-*R*, *MGC*157263, *MTHFSD*, *MVD*, *MYLK*3, *NETO*2, *ORC*6, *PABPN*1*L*, *PIEZO*1, *RNF*166, *RPL*13, *SHCBP*1, *SNAI*3, *SPATA*2*L*, *SPG*7, *SPIRE*2, *TCF*25, *TRAPPC2L*, *TUBB*3, *VPS*35, *VPS9D*1, *ZC3H*18, *ZCCHC*14, *ZFPM*1, *ZNF*276, *ZNF*469
rs29014958	18	*AARS*, *ADAT*1, *BCAR*1, *BCNT*, *CFDP*1, *CNTNAP*4, *COG*4, *CTRB*1, *DDX*19*A*, *DDX*19*B*, *EEF*1*A*1, *FA2H*, *FUK*, *GABARAPL*2, *GLG*1, *IL*34, *KARS*, *LDHD*, *MLKL*, *MRCL*, *MTSS*1*L*, *P*97*BCNT*2, *PDPR*, *RFWD*3, *SF3B*3, *SIAT4B*, *TERF*2*IP*, *TMEM*170*A*, *TMEM*231, *UQCRFS*1, *VAC*14, *VSTM2B*, *WDR*59, *ZFP*1, *ZNRF*1
rs29020544	18	*ANKRD*27, *CEBPG*, *CEP*89, *CHST*8, *DPY*19*L*3, *FAAP*24, *GPATCH*1, *GPI*, *KCTD*15, *KIAA*0355, *LRP*3, *LSM*14*A*, *NUDT*19, *PDCD2L*, *PDCD*5, *PEPD*, *RGS9BP*, *RHPN*2, *SLC7A*10, *SLC7A*9, *TDRD*12, *TSHZ*3, *UBA*2, *WDR*88, *WTIP*, *ZNF*507
rs41578926	18	*MON*1*B*, *SYCE*1*L*
rs41257403/ rs41636878	19	*AATK*, *ACTG*1, *ALYREF*, *ANAPC*11, *ARHGDIA*, *ARL*16, *ASPSCR*1, *AZI*1, *BAIAP*2, *BPTF*, *C*17*orf*62, *C*17*orf*70, *C*1*QTNF*1, *CANT*1, *CARD*14, *CBX*2, *CBX*4, *CBX*8, *CCDC*137, *CCDC*40, *CCDC*47, *CCDC*57, *CD*7, *CD*79*B*, *CEP*95, *CHMP*6, *CSNK*1*D*, *CYTH*1, *DCXR*, *DDX*42, *DDX*5, *DNAH*17, *DUS*1*L*, *EIF4A*3, *ENDOV*, *ENGASE*, *ENPP*7, *ENTHD*2, *ERN*1, *FAM*195*B*, *FASN*, *FN*3*K*, *FN3KRP*, *FOXK*2, *FSCN*2, *FTSJ*3, *GAA*, *GCGR*, *GH*1, *GPS*1, *HEXDC*, *HGS*, *ICAM*2, *KPNA*2, *LGALS3BP*, *LRRC*45, *MAFG*, *METRNL*, *MILR*1, *MRPL*12, *MYADML*2, *NARF*, *NOL*11, *NPB*, *NPLOC*4, *NPTX*1, *OGFOD*3, *OXLD*1, *P4HB*, *PCYT*2, *PDE6G*, *PGS*1, *POLG*2, *PPP*1*R*27, *PSMC*5,*PSMD*12 ,*PYCR*1 ,*RAB*40*B* , *RAC*3, *RBFOX*3, *RFNG*, *RNF*213, *RPTOR*, *SCN4A*, *SECTM*1, *SGSH*, *SIRT*7, *SLC*16*A*3, *SLC*26*A*11, *SLC*38*A*10, *SMARCD*2, *SMURF*2,*STRA*13 , *STRADA*, *TBC*1*D*16, *TBCD*, *TEX*2, *TIMP*2, *TSPAN*10, *USMG*5, *USP*36, *UTS2R*, *WDR*45*L*,*ZNF*750
rs109838275	20	*ADAMTS*16, *ADCY*2, *IRX*1, *KIAA*0947, *MED*10, *NSUN*2, *PAPD*7, *SRD5A*1,
rs41577870	20	*CDH*10, *CDH*9

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rs41628334/ rs41639270/rs41639268/ rs41940571/rs29021255/ rs41942930/ rs41578305	20	*ADAMTS*12, *AGXT*2, *AMACR*, *BRIX*1, *C1QTNF*3, *C20H5ORF*22, *C20H5orf*34, *C5orf*28, *C5orf*42, *C5orf*51, *C6*, *C7*, *C9*, *CAPSL*, *CARD6*, *CCDC*152, *CCL28*, *CDH6*, *DAB2*, *DNAJC21*, *DROSHA*, *EGFLAM*, *EMB*, *FBXO*4, *FYB*, *GDNF*, *GHR*, *GOLPH*3, *HCN*1, *HMGCS*1, *IL7R*, *LIFR*, *LMBRD*2, *MROH2B*, *MRPS*30, *MTMR*12, *NADK*2, *NIM*1K, *NIPBL*, *NNT*, *NPR3*, *NUP*155, *OSMR*, *OXCT*1, *PAIP*1, *PARP8*, *PLCXD*3, *PRKAA*1, *PRLR,PTGER*4, *RAD*1, *RAI*14, *RANBP3L*, *RICTOR*, *RPL37*, *RXFP3*, *SKP2*, *SLC*1A3, *SLC45A*2, *SPEF*2, *SUB*1, *TARS*, *TTC23L*, *TTC33*, *UGT3A*1, *WDR70*, *ZFR*, *ZNF*131
rs41654340	23	*ABT*1, *ALDH5A*1, *BTN*1A1, *C23H6ORF62*, *DCDC2*, *FAM65B*, *GMNN*, *GPLD*1, *H2B*, *HFE*, *HIST*1H1A, *HIST*1H1C, *HIST*1H1D, *HIST*1H1E, *HIST*1H2AC, *HIST*1H2AD, *HIST*1H2AG, *HIST*1H2AJ, *HIST*1H2BA, *HIST*1H2BB, *HIST*1H2BI, *HIST*1H2BJ, *HIST*1H2BK, *HIST*1H3A, *HIST*1H3B, *HIST*1H3E,HIST1H3H, *HIST2H4B*, *HIST4H4*, *HMGN4*, *KIAA*0319, *LRRC16A*, *MGC*157324, *MRS*2, *NKAPL*, *OR12D3*, *OR14J*1, *OR2B6*, *PGBD*1, *POM*121L2, *PRSS*16, *SCGN*, *SLC*17A1, *SLC*17A2, *SLC*17A3, *SLC*17A4, *TDP2*, *THEM*2, *TRIM27*, *TRIM38*, *ZNF*165, *ZNF*184, *ZNF*192, *ZNF*311, *ZNF*323, *ZNF*391, *ZSCAN*12, *ZSCAN*16, *ZSCAN*9
BFGL-NGS-119848	25	*ATF7IP*2, *BCAR4*, *CIITA*, *CLEC16A*, *CPPED*1, *DEXI*, *EMP2*, *ERCC4*, *GSPT*1, *LITAF*, *MKL*2, *NUBP*1, *PRM*1, *PRM2*, *PRM3*, *RMI2*, *RSL1D*1, *SHISA*9, *SNN*, *SNX29*, *SOCS*1, *TEKT5*, *TNP2*, *TVP23A*, *TXNDC*11, *ZC3H7A*
Hap-map25382-BTC-000577	25	*ADCY9*, *ALG*1, *ANKS3*, *C16orf*89, *C25H16ORF*71, *CDIP*1, *CORO7*, *DNAJA3*, *FAM86A*, *GLIS2*, *GLYR*1, *HMOX2*, *MGC*142811, *MGRN*1, *NAGPA*, *NMRAL*1, *NUDT*16L1, *PAM*16, *PPL*, *RBFOX*1, *ROGDI*, *SEC14L5*, 12-*Sep*, *SRL*, *TFAP*4, *UBN*1, *VASN*, *ZNF*500
rs41606777	26	*ATRNL*1, *C10orf*82, *CACUL*1, *CCDC*172, *EIF3A*, *EMX*2, *ENO4*, *FAM204A*, *FAM45A*, *GFRA*1, *GRK5*, *HSPA12A*, *KCNK*18, *KIAA*1598, *PDZD8*, *PNLIP*, *PNLIPRP2*, *PNLIPRP3*, *PRDX3*, *PRLHR*, *RAB*11FIP2, *SFXN*4, *SLC*18A2, *VAX*1
rs41650611	26	*ALDH*18A1, *ANKRD2*, *AVPI*1, *BLNK*, *C10orf*12, *CCNJ*, *CRTAC*1, *CYP2C*18, *CYP2C*19, *CYP2C*87, *DNTT*, *ENTPD*1, *EXOSC*1, *FRAT*1, *FRAT2*, *GOLGA7B*, *HELLS*, *HOGA*1, *HPS*1, *LCOR*, *LOXL*4, *MARVELD*1, *MMS*19, *MORN*4, *NOC3L*, *OPALIN*, *PDLIM*1, *PGAM*1, *PI4K2A*, *PIK3AP*1, *PLCE*1, *PYROXD*2, *R3HCC*1L, *RRP*12, *SFRP5*, *SLIT*1, *SORBS*1, *TBC*1D12, *TCTN*3, *TLL2*, *TM9SF*3, *UBTD*1, *ZDHHC*16, *ZFYVE*27
rs41648482	28	*ACTN2*, *ARID4B*, *B3GALNT*2, *EDARADD*, *ERO*1LB, *GGPS*1, *GNG4*, *GPR137B*, *HEATR*1, *IRF2BP*2, *KCNK*1, *LGALS8*, *LYST*, *MTR*, *NID*1, *RBM34*, *RYR2*, *SLC35F*3, *TARBP*1, *TBCE*, *TOMM*20
rs29027496	29	*ADRBK*1, *AIP*, *ALDH3B*1, *ALDH3B*2, *ANKRD13D*, *AP5B*1, *ARL*2, *ATG2A*, *ATL3*, *B3GNT*1, *BAD*, *BANF*1, *BATF2*, *BRMS*1, *C11orf*80, *C11orf*85, *C29H11orf*68, *C29H11orf*84, *C29H11orf*86, *CABP2*, *CABP4*, *CAPN*1, *CARNS*1, *CATSPER*1, *CCDC85B*, *CCDC*87, *CCDC88B*, *CCS*, *CD*248, *CDC42BPG*, *CDC42EP*2, *CDCA5*, *CDK2AP*2, *CFL*1, *CHKA*, *CLCF*1, *CORO*1B, *CST6*, *CTSF*, *CTSW*, *DNAJC*4, *DOC2G*, *DPF2*, *DPP3*, *DRAP*1, *EFEMP*2, *EHBP*1L1, *EHD*1, *EIF*1AD, *ESRRA*, *FAU*, *FERMT3*, *FIBP*, *FKBP*2, *FLRT*1, *FOSL*1, *FRMD8*, *GAL3ST*3, *GPHA*2, *GPR*137, *GPR*152, *GSTP*1, *KAT5*, *KCNK*4, *KCNK*7, *KDM2A*, *KLC*2, *LGALS*12, *LRFN*4, *LTBP*3, *MACROD*1, *MAP3K*11, *MAP4K*2, *MARK*2, *MEN*1, *MGC*140823, *MRPL*11, *MRPL*49, *MUS*81, *NAALADL*1, *NAT*11, *NDUFS8*, *NDUFV*1, *NPAS4*, *NRXN2*, *NUDT22*, *NUDT8*, *OTUB*1, *OVOL*1, *PACS*1, *PC*, *PCNXL*3, *PELI*3, *PITPNM*1, *PLA2G*16, *PLCB3,POLA2 ,POLD*4 *,PPP2R5B*, *PRDX5*, *PTPRCAP*, *PYGM*, *RAB*1B, *RAD9A*, *RASGRP2*, *RBM*14, *RBM*4, *RBM4B*, *RCE*1, *RCOR2*, *RELA*, *RHOD*, *RIN*1, *RNASEH2C*, *RPS6KA*4, *RPS6KB*2, *RTN3*, *SAC3D*1, *SART*1, *SCYL*1, *SF*1, *SF3B*2, *SIPA*1, *SLC22A*11, *SLC22A*12, *SLC25A*45, *SLC29A*2, *SNX*15, *SNX*32, *SPDYC*, *SPTBN*2, *SSH3*, *SSSCA*1, *STIP*1, *SUV420H*1, *SYT*12, *SYVN*1, *TBC*1D10C, *TBX*10, *TCIRG*1, *TIGD*3, *TM7SF*2, *TMEM*134, *TMEM*151A, *TRMT*112, *TRPT*1, *TSGA*10IP, *UNC*93B1, *VEGFB*, *VPS*51, *YIF*1A, *ZDHHC*24, *ZFPL*1, *ZNHIT*2
rs41608052	29	*ARHGAP32*, *BARX*2, *ETS*1, *FLI*1, *IGSF9B*, *JAM*3, *KCNJ*1, *KCNJ*5, *NTM*, *OPCML*, *SPATA*19
Hap-map42724-BTA-91683	X	*ACTRT*1, *AIFM*1, *APLN*, *BCORL*1, *CCDC*169, *CXHXorf*64, *ELF4*, *GPR*119, *IRF2BP*1, *OCRL*, *RAB33A*, *RBMX*2, *SASH3*, *SLC25A*14, *SMARCA*1, *UTP*14A, *XPNPEP2*, *ZDHHC*9, *ZNF*280C
rs109224945/ BTA-94079-no-rs	X	*CASK*, *DDX*3X, *DUSP*21, *FUNDC*1, *GPR*34, *GPR*82, *MAOA*, *MAOB*, *MED*14, , *NDP*, *NYX*, *USP*9Y
rs41629005	X	*AFF*2, *CXORF*23, *FMR*1, *FMR*1NB, *IDS*, *MAP7D*2, *SLITRK*2

[#]Gene selection is based on UMD 3.1 genome assembly.
[+]Underscore denotes SNPs which appear in different GWAS files.

Table S5. IPA function analysis on the 1635[#] candidate genes selected from genome-wide association study with SCC in dairy cows.

| Name	Molecules
Diseases and Disorders	
Cancer	1130
Gastrointestinal Disease	526
Inflammatory Response	73
Hepatic System Disease	17
Infectious Disease	24
Molecular and Cellular Functions	
Cell-To-Cell Signalling and Interaction	76
Cellular Movement	76
Energy Production	8
Small Molecule Biochemistry	42
Cell Morphology	254
Physiological System Development and Function	
Haematological System Development and Function	181
Immune Cell Trafficking	53
Tissue Development	150
Auditory and Vestibular System Development and Function	10
Organ Morphology	105

[#]1568 Genes with known IPA-eligible human and rodent orthologs were considered.

Table S6. Top 10 signalling pathways from IPA among the 1635[#] candidate genes selected from genome-wide association study with SCC in dairy cows.

Canonical pathways[*]	Molecules	Ratio
Agranulocyte Adhesion and Diapedesis	28	1.47E−01
Granulocyte Adhesion and Diapedesis	25	1.4E−01
NAD Phosphorylation and Dephosphorylation	6	3E−01
Proline Biosynthesis I	5	3.57E−01
Role of JAK2 in Hormone−like Cytokine Signalling	8	2.29E−01
IL−17A Signalling in Gastric Cells	6	2.4E−01
Protein Kinase A Signalling	41	1.04E−01
Remodelling of Epithelial Adherens Junctions	11	1.62E−01
Serotonin Degradation	12	1.54E−01
Ethanol Degradation II	8	1.86E−01

[*]All pathways with P < 0.01
[#]1568 genes with known IPA-eligible human and rodent orthologs were considered.
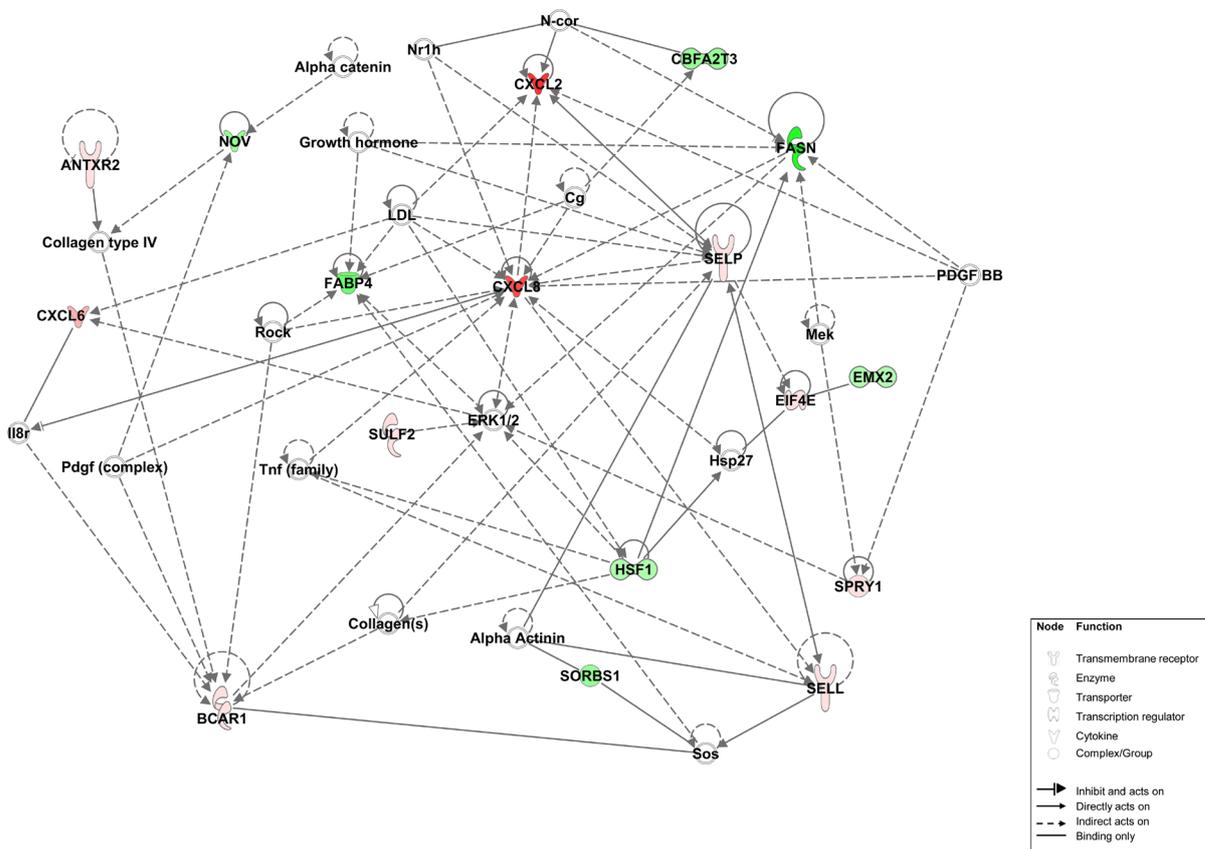
Figure S1. IPA Network 1 based on candidate genes for mastitis involved in Cancer, Organismal Survival, Cellular Movement. Red denotes up-regulation and green down-regulation of the gene. The shapes of nodes indicate different types of gene product as shown in the insert. Note that although the pathway is classified in IPA under "cancer" the same genes are also involved in proliferation and migration of normal cells and are thus relevant to the cellular changes to infection in the bovine mammary gland.