MINING FOR THE ASSOCIATION OF BOVINE MASTITIS LINKED GENES TO PATHOLOGICAL SIGNATURES AND PATHWAYS

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Abstract

Background: Bovine mastitis is a common infectious disease with a serious threat to the dairy industry and public health. Mastitis is a polygenetic trait under the control of many genes. In the current study, our research attempted to address the role of mastitis-associated genes in various signalings including parasitic, viral, cancer and fungal diseases by using online bioinformatics software. **Methods:** We selected mastitis-associated genes from already published data and using online bioinformatics tools including DAVID and String classified the pathological role of relevant genes. A Venn diagram was used to show the status of overlapping genes among different biological function processes. **Result:** This study revealed that the genes gathered in published resources of mastitis were significantly correlated with influenza A, chagas disease, leishmaniasis, toxoplasmosis, tuberculosis, cancer signaling, hepatitis B, type I & II diabetes mellitus and prion diseases biological pathways. Based on our findings, we concluded that mastitis-linked genes could be used as markers for many other diseases. Moreover, the bioinformatics tools applied in the current study might be helpful in screening the genes involved in one disease and their association with other diseases as well.

Key words: bovine mastitis, bioinformatics tools, biological signaling, genetic markers

Bovine mastitis, the inflammation of udder tissue, is associated with physical and chemical changes in milk and has been classified as an important disease in the dairy cow industry (Chen et al., 2019). Due to high culling rates, milk losses, increased treatment cost, labor costs, and negative influences on human health, bovine mastitis is considered a serious threat to the dairy industry (Gomes and Henriques, 2016). Furthermore, mastitis is an immunity and inflammatory-related disease controlled by several genes (Thompson-Crispi et al., 2014; Griesbeck-Zilch et al., 2009). The susceptibility of dairy animals to udder infection is heritable (Tolone et al., 2016); however, limited knowledge about the complex physiological and cellular processes that occur in the mammary gland in response to pathogens is available. Thus, today, researchers are more interested in identifying the genetic markers for disease susceptibility and selection of mastitis-resistant breeds (Spaan et al., 2013).

Transcriptome analysis is considered a powerful tool to pinpoint several aspects of mastitis including the molecular mechanism and disease pathology (Wiggans et al., 2017). On the other hand, transcriptomic screening

is also performed to identify the genes involved in the disease development, interaction of these genes during disease pathogenesis and their regulated biological signaling linked with the progression of infection (Jensen et al., 2013). Identifying the commonalities between mastitis and other diseases will provide clues for the therapeutic targets for mastitis and other diseases as well. Several RNA-Seq and microarray datasets are available online and utilizing these datasets for genetic analysis will help us to screen the genetic markers and related signalings. Moreover, full knowledge regarding protein function and their specific interaction during disease progression is warranted (Lee et al., 2007). In this regard, String online-based software is widely in practice to establish the interaction of proteins with each other during disease progression (Szkłarczyk et al., 2015). In the current research we utilized some online software such as String, DAVID and GO for the mastitis-associated genes and noticed that these molecular signatures are also involved in the regulation of other parasitic, bacterial and viral related diseases signaling. Thus the current findings provide a baseline for already reported genes in mastitis and their rules in the regulation of other disease signalings.

Table 1. List of mastitis associated significantly regulated genes selected from GWAS, RNA-Seq, microarray, Chip-assay and meta-analysis studies

Mastitis associated genes	Author	Technique
1	2	3
ELMO1, ASB4, DPYS, EPGN, FGF6, HPN, LOC786512, NFKB1, PDE6C, RORA, PDI46, ESRI, HES2, NFKB1, SLC2A13, SMYD3, TMEM130, ADAMTSL1, BMP7, CCDC77, CHD5, CORO7, CUX1, DUOX1, MGAT4A, NPFFR2, NRDC, PARM1, PLCXD3, PLPP3, PON1, PPARD, PPARD10, RAB3B, RORA, SP2, TBX20, TXN, ZFAND3, CD14, CFB, ALOX5, ALOX54P, ALPL, MPD3, ARL10ATP12A, CCL2, CCL20CCL5, CX3CR1, CXCL10, CXCL8, CXCR1, DUSP4, ESPNL, FOS, GCA, GPAT2, HSPB8, IL-8, JUNB, JUNC, LTBP4, MYD88, NF-KB, PAD14, JAK2, DUSP4, CD4, PIM1, PTAFR, SLC13A5, SOCS3, TGFB1, TGM3, TLR4, TNFAIP3, TNFA, STAT5A, STAT5B, ZC3H12, ZNF683, IL10, ARG1, CAST, CST3, GHR, KLRK1, NF200, TPM, TPM2, TPM3, TPM4, CCR6, HOXA6, IL13RA1, TPM1, IL8R, OPN, PGLYRP-1, SELL, TLR2, A2M, APM1, CREB1, CXCL14, F4BP3, F4DS1, F4M62C, GLI2, HSPD1L8R8, INHBB, MAP3K11, PEKL, RBP1, STA6GAL, STMN1, TP73L, UB45, HIST1H2BN, MST1, NRGI, SLC27A5, CCPGI, CXCL12, DEFB5, ECHDC1, FBXL8, GATAD1, HP1, IDH1, NRGI, LGALS4, LLGL1, MAD2L1B, MRPS12, MST1, NAT9, P2RY12, RBM18, RGS9, XDH, TRAPP9, THAP6SLC39A9, RUND34, SLC31A2, Casp1, CCR8, CP-2, CTLA4, FcR2, FcRN, GH, ICAM3, IFN1, IFN1, IKBKG, IL10, IL12A, IRAK1, IRAK4, IRF3, IRF5, IRF7, JUN, MAP2K2, LTb, MCP-1, M-CSFb, MHC-1, MIF, MSHR, SOD, RANTES, SUG1, TAP1, TAP2, TcRb, TcRd, TCRg, TCRz, A2M, ARHGAP39, DGAT1, PTK2, MAST3, PDGFD, PTX3, STAB2, AKT1, CASP8, IFNAC, IFNAR2, IFNW1, IKBKG, IL10, IL12A, IRAK1, IRAK4, IRF3, IRF5, IRF7, JUN, MAP2K2, MAP2K3, MAP2K6, MAP3K7, MAPK10, MAPK13, MAPK14, MAPK8, TLR8, TLR9, TLR7, TLR1, TLR3, TLR6, PIK3CA, TNF, TICAM2, PIK3CD, RELA, STAT1, TAB2, TBK1, ACSL6, ATF3, BCL2A1, BIRC3, BTG2, CCL11, CCL2, CCL3L1, CCRI, CD40, CDKN1A, CSF3, CSF3RCXCL1, CXCL2, EGR1, FGD6, FUT8, HCK, HSPA1A, IER3, IFNG, IL11RA, IL6, IL8, LEP, MARCKS, MARCKSL1, MGC21881, MTMR10, NFKB2, NOS2A, NR2F6, PLAUR, S100A2, SLC24, S100A12, SOD2, TIMP1, THBS1, TNFRSF9	Cai et al., 2018 ----- ----- Fang et al., 2016 ----- ----- ----- ----- He et al., 2016 Jensen et al., 2013 Karthikeyan et al., 2016 Wang et al., 2014 Ogorevc et al., 2009 ----- Song et al., 2016 ----- ----- Tao and Mallard, 2007 ----- Wang et al., 2015 Welderufael et al., 2018 Wu et al., 2015 ----- ----- Younis et al., 2016 ----- -----	RNA-Seq ----- ----- RNA-Seq ----- ----- ----- ----- ChIP-seq Microarray GWAS study DNA-seq GWAS study ----- DNA-methylome ----- ----- Microarray ----- GWAS study GWAS study Gene-chip assay ----- ----- Meta-analysis ----- -----

<i>TUBA1, WARS, ZFYVE9</i>	-----	-----	-----
<i>ACACA, ACSM1, AGPAT1, AGPAT6d, ALOX5, BCAT2, BCL2, BNBD-9, BOLA-RD4, C1S, C2, C3, CBS, CCL3, CCL4</i>	-----	-----	-----
<i>CCR5, COQ2, COTL1, CXCL11, CXCL16, CYBA, DEFB10, DEFB44, FASN, FCER1G, FDFIT1, FGR, FN1, GP4M, IL1A</i>	-----	-----	-----
<i>HMGCR, HMOX1, IL1B, ITGB6, LAP, LBP1, LOC504773, LPL, LTF, LTA4H, LY22, MVK, NCF1, NFKBIZ, NOS2, ORL1</i>	-----	-----	-----
<i>ORM1, PEMT, PSAT1, PYCRI, RAB27R, RSAD2, VNN1, TM7SF2, THBS1, TAPI, SELP, SAA3, SCD, SERPINF2</i>	-----	-----	-----
<i>BCL2, MAPK1, MAPK14, AKT1, MAPK3, MAPK8, MAPK14, BCL2, NFKBIZ, IL8, CXCL2, CCL20, CFB, S100A9</i>	Sharifi et al., 2018, 2019	Meta-analysis	
<i>BRCA1, MBL1</i>	Yuan et al., 2012, 2013	DNA-Seq	
<i>CXADR, GRO1, CXCL2, MAZ, FOXO4, LEFT1, ERRI, AP4, MET, E12, NFAT, KPN1, SMARCA4, ABL1,</i>	Han, 2019	Microarray	
<i>KRT8, BRD4, NAT9, MST1, NRGI, MAZ, AP4, SPI, AP4</i>	Han, 2019	Microarray	

Material and methods

Data collection and Gene's selection

The genes significantly ($P < 0.05$) regulated (down- and up-regulated) in bovine mastitis reported by previous studies were selected for the current study (Table 1). In addition, the inflammation and immunity-related genes documented in mastitis were selected from meta-analysis studies (Sharifi et al., 2018, 2019; Younis et al., 2016). Moreover, the above-selected genes were analyzed for their association with other diseases using already well-established online software. All published studies that have discussed the mastitis-associated genes were screened through authentic sources including PubMed, ScienceDirect, Web of Science, SpringerLink, Scopus and Google Scholar (Khan et al., 2020). The major keywords used for the search of literature were bovine mastitis, mastitis linked genetic markers, GWAS studies, transcriptomic responses, DAVID, KEGG, GO, string, KOBAS and Venn diagram. Moreover, the data published only in the English language and well-reputed peer-reviewed journals have been included in the current study. Furthermore, we excluded all content available in the form of conference abstracts, books, book chapters and unpublished findings.

Bioinformatic analysis

The functional analysis of bovine mastitis-associated genes was carried out using KEGG biological pathways (KOBAS2.0) and DAVID (<https://david.ncifcrf.gov/>) (Huang et al., 2009). The association of signaling path-

way was counted significant for which P -value < 0.05 . The String is a web-based tool that is used for the mining of protein-protein interaction (Szkarczyk et al., 2015). The list of the mastitis-associated genes selected from already published studies was uploaded on String and run to analyze protein-protein interaction with the highest confidence level (0.9). A maximum number of interactions to show were set up with none (first and second shell) (Szkarczyk et al., 2015).

Results

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of selected genes by using a database for annotation, visualization, and integrated discovery (DAVID) bioinformatics resources

Our findings revealed that bovine mastitis-linked genes significantly alter genes signature involved in several pathological conditions signaling as mentioned in Figure 1. The genes involved in different pathways have been provided in Table 2. In addition, our results showed that the bovine mastitis-associated genes significantly regulated influenza A, Chagas disease, toxoplasmosis, leishmaniasis and pertussis linked signaling. The level of significance for each signaling regulated by mastitis-associated genes is summarized in Table 2. Consistently, our result showed that *NFKB1A*, *JAK2*, *MAPK*, *STATs*, *RELA*, *MYD88*, *TNF*, *FOS*, *TLR2*, *TLR4*, *AKT1*, *NFKB1*, *IL1A*, *IL1B* and *JUN* were involved in the majority of other disease signalings (Table 2).

Figure 1. Bovine mastitis association genes regulated other disease pathways such as influenza A, Chagas, toxoplasmosis, anaplasmosis, salmonella infection and prion diseases

Note: DAVID, web based tool was performed for the screening of other disease signaling pathways regulated by mastitis linked genes and all the significantly (P -value < 0.05) regulated disease linked pathways were considered

Table 2. The KEGG pathway analysis revealed that bovine mastitis linked genes regulated parasitic (toxoplasmosis, leishmaniasis, Chagas disease etc), bacterial (Salmonella infection, tuberculosis) and viral (influenza A, hepatitis C, measles) disease linked signaling pathways

Biological pathways	Total genes	P-value	Genes
Influenza A	46	1.45E-31	<i>IFNAC, TNF, CCL2, TBK1, IL18, TLR3, NFKBIA, RSAD2, CXCL8, HSPA1A, TLR4, NFKB1, CCL5, TLR7, CXCL10, IRAK4, AKT1, MYD88, IFNG, PYCARD, PIK3CA, IL1B, CASP1, MAP2K6, IL1A, IL6, MAP2K2, SOCS3, MAP2K3, RELA, PIK3CD, MAPK11, MAPK10, STAT1, IKBKE, IFNAR2, MAPK13, IRF7, JUN, MAPK14, IL12A, IRF3, JAK2, MAPK8</i>
Chagas disease	36	2.62E-27	<i>CCL3, CCL2, TNF, C3, TLR2, NFKBIA, CXCL8, TLR4, NFKB1, CCL5, TLR6, TGFB1, IL10, TLR9, IRAK4, AKT1, FOS, MYD88, CASP8, IFNG, PIK3CA, IL1B, NOS2, IRAK1, IL6, RELA, PIK3CD, MAPK11, MAPK10, MAPK13, JUN, MAPK14, IKBKG, IL12A, MAPK8</i>
Toxoplasmosis	35	3.26E-26	<i>TNF, TLR2, NFKBIA, HSPA1A, NFKB1, TLR4, IL10, TGFB1, MAP3K7, AKT1, IRAK4, MYD88, BCL2, CASP8, IFNG, NOS2, MAP2K6, IRAK1, MAP2K3, RELA, MAPK11, MAPK10, CD40, BIRC3, STAT1, TAB2, CCR5, MAPK13, MAPK14, IKBKG, IL12A, JAK2, MAPK8, ALOX5</i>
Leishmaniasis	29	1.81E-25	<i>TNF, MARCKSL1, C3, TLR2, NFKBIA, NFKB1, TLR4, IL10, TGFB1, MAP3K7, IRAK4, FOS, MYD88, IFNG, IL1B, NOS2, IL1A, IRAK1, NCF1, RELA, MAPK11, STAT1, TAB2, CYBA, MAPK13, JUN, MAPK14, IL12A, JAK2</i>
Pertussis	29	2.63E-24	<i>TNF, C3, CXCL8, NFKB1, TLR4, C1S, IL10, IRAK4, FOS, MYD88, TICAM2, PYCARD, IL1B, NOS2, C2, CASP1, IL1A, IRAK1, IL6, RELA, MAPK11, MAPK10, MAPK13, JUN, MAPK14, IL12A, IRF3, MAPK8, CD14</i>
Measles	35	7.32E-23	<i>IFNAC, TBK1, STAT5A, STAT5B, TLR2, NFKBIA, HSPA1A, NFKB1, TLR4, TLR7, TLR9, MAP3K7, IRAK4, AKT1, MYD88, IFNG, PIK3CA, IL1B, IL2RG, IL1A, IRAK1, IL6, RELA, PIK3CD, STAT1, TAB2, IKBKE, IFNAR2, IRF7, IL12A, IRF3, JAK2, TNFAIP3</i>
Tuberculosis	38	5.43E-22	<i>IFNAC, TNF, C3, IL18, TLR1, TLR2, TLR4, NFKB1, TLR6, TGFB1, IL10, TLR9, IRAK4, AKT1, MYD88, BCL2, CASP8, IFNG, IL1B, FCER1G, NOS2, LBP, IL1A, IRAK1, IL6, CREB1, RELA, MAPK11, MAPK10, STAT1, MAPK13, MAPK14, IL12A, JAK2, MAPK8, HSPD1, CD14</i>
Hepatitis B	34	6.35E-21	<i>IFNAC, TNF, TBK1, STAT5A, STAT5B, TLR2, TLR3, NFKBIA, CXCL8, NFKB1, TLR4, TGFB1, AKT1, FOS, MYD88, BCL2, CASP8, TICAM2, PIK3CA, IL6, MAP2K2, CREB1, RELA, PIK3CD, MAPK10, STAT1, IKBKE, CDKN1A, IRF7, JUN, IKBKG, IRF3, MAPK8</i>
Legionellosis	23	5.82E-20	<i>IL6, TNF, C3, IL18, RELA, CXCL2, TLR2, CXCL8, NFKBIA, HSPA1A, NFKB1, TLR4, NFKB2, MYD88, CASP8, PYCARD, IL12A, IL1B, HSPD1, CASP1, CD14</i>
Salmonella infection	26	1.72E-19	<i>CCL3, IL18, CXCL2, CXCL8, NFKB1, TLR4, CCL4, FOS, MYD88, IFNG, PYCARD, IL1B, NOS2, LBP, CASP1, IL1A, IL6, RELA, MAPK11, MAPK10, MAPK13, JUN, MAPK14, MAPK8, CD14</i>
Herpes simplex infection	35	2.46E-18	<i>IFNAC, CCL2, TNF, C3, TBK1, TLR2, TLR3, NFKBIA, NFKB1, CCL5, TLR9, MAP3K7, FOS, MYD88, TAP2, CASP8, TAP1, IFNG, IL1B, IL6, SOCS3, RELA, MAPK10, STAT1, TAB2, IKBKE, IFNAR2, IRF7, JUN, IKBKG, IL12A, IRF3, JAK2, MAPK8</i>
Malaria	19	2.35E-15	<i>CSF3, SELP, IL6, CCL2, TNF, IL18, TLR2, KLRK1, CXCL8, TLR4, CD40, IL10, TGFB1, TLR9, MYD88, IFNG, IL12A, IL1B, THBS1</i>
Hepatitis C	25	3.03E-13	<i>IFNAC, TNF, TBK1, CXCL8, TLR3, NFKBIA, NFKB1, AKT1, PIK3CA, IL6, SOCS3, RELA, PIK3CD, MAPK11, MAPK10, STAT1, IKBKE, IFNAR2, CDKN1A, MAPK13, IRF7, MAPK14, IKBKG, MAPK8, IRF3</i>
Inflammatory bowel disease (IBD)	19	5.49E-13	<i>IL6, TNF, IL18, RELA, TLR2, NFKB1, TLR4, RORA, STAT1, TGFB1, IL10, IL12RB2, IL17A, JUN, IFNG, IL12A, IL1B, IL2RG, IL1A</i>
Rheumatoid arthritis	10	1.53E-11	<i>IL6, CCL3, CCL2, TNF, IL18, CTLA4, TLR2, CXCL8, TLR4, CCL5, CXCL12, TGFB1, FOS, IL17A, CCL20, JUN, IFNG, IL1B, LTB, IL1A</i>
Amoebiasis	19	2.31E-09	<i>IL6, TNF, RELA, PIK3CD, TLR2, CXCL8, NFKB1, TLR4, TGFB1, IL10, ARG1, PTK2, IFNG, IL12A, IL1B, PIK3CA, NOS2, CD14, FNI</i>
African trypanosomiasis	9	7.70E-06	<i>IL6, TNF, MYD88, IL18, IFNG, IL12A, IL1B, IL10, TLR9</i>
Prion diseases	6	0.002083	<i>EGR1, IL6, MAP2K2, IL1B, CCL5, IL1A</i>
Type II diabetes mellitus	6	0.00221	<i>TNF, IFNG, IL12A, IL1B, HSPD1, IL1A</i>
Type I diabetes mellitus	7	0.016861	<i>TNF, SLC2A4, SOCS3, PIK3CD, PIK3CA, MAPK8, MAPK10</i>
Asthma	5	0.021511	<i>CCL11, TNF, FCER1G, CD40, IL10</i>

Association of bovine mastitis associated genes with cancer signalings

We have also mined to confirm that mastitis-linked genes were associated with cancer signalings (Figure 2). In line with our expectations, bovine mastitis-linked genes regulate signaling pathways known to be important in cancers like prostate, pancreatic, colorectal cancer, melanoma, etc. The genes involved in different types of cancer pathways have been summarized in Table 3.

Protein-protein interaction networks of differentially expressed genes

We also visualized the protein-protein network using String. The String analysis showed that the mastitis

linked-genes network is merged at *TLR* signaling via *MYD88* or concentrating on chemokine signaling. Another branch of signaling pathways is originating from JAK2/Stat1 or MAP kinases (Figure 3).

The list of genes involved in immune response, inflammatory signaling, LPS signaling and innate immunity has been provided in Figure 4. We further confirm the overlapping of several signaling pathways that share commonality with mastitis, as demonstrated in the Venn diagram. Here we observed that all the signaling pathways share a degree of similarity; however, none of the genes was common in all pathways (Figure 5).

Bovine mastitis associated genes involve in cancer signaling pathways

Figure 2. Bovine mastitis linked genes involved in regulation of cancer signaling pathways

Note: DAVID, web based tool was used to analyse the correlation and significant cut was made below P-value 0.05

Table 3. The KEGG pathway analysis revealed that Bovine mastitis linked genes regulated cancer linked signaling pathways (prostate cancer, colorectal cancer, pancreatic cancer, small cell lung cancer etc.,)

Biological pathways	Total genes	P-value	Genes
Prostate cancer	13	6.04E-06	<i>MAP2K2, CREB1, RELA, PIK3CD, NFKBIA, LEF1, NFKB1, AKT1, CDKN1A, BCL2, IKBKG, PIK3CA, PDGFD</i>
Chronic myeloid leukemia	12	6.96E-06	<i>AKT1, CDKN1A, MAP2K2, STAT5A, RELA, IKBKG, PIK3CD, STAT5B, NFKBIA, PIK3CA, NFKB1, TGFB1</i>
Pancreatic cancer	10	9.60E-05	<i>AKT1, RELA, IKBKG, PIK3CD, PIK3CA, NFKB1, MAPK8, MAPK10, STAT1, TGFB1</i>
Colorectal cancer	10	1.08E-04	<i>AKT1, FOS, BCL2, JUN, PIK3CD, LEF1, PIK3CA, MAPK8, MAPK10, TGFB1</i>
Melanoma	8	0.004671	<i>AKT1, FGF6, CDKN1A, MAP2K2, PIK3CD, MET, PIK3CA, PDGFD</i>
Renal cell carcinoma	7	0.011872	<i>AKT1, MAP2K2, JUN, PIK3CD, MET, PIK3CA, TGFB1</i>
Small cell lung cancer	12	3.84E-05	<i>AKT1, PTK2, BCL2, RELA, IKBKG, PIK3CD, NFKBIA, PIK3CA, NFKB1, NOS2, BIRC3, FN1</i>

Figure 3. Protein-protein interaction network of genes produced by STRING analysis. The colored lines represent seven types of evidence used in predicting associations. Red line: fusion evidence; blue line: co-occurrence evidence; yellow line: text mining evidence; green line: neighbourhood evidence; purple line: experimental evidence; light blue line: database evidence; and black line: co-expression evidence

Figure 4. The genes majorly associated with LPS mediated signaling, inflammatory, immune and innate immune responses

Figure 5. Venn diagram shows the status of overlapped genes associated with LPS mediated signaling, inflammatory, immune response and innate immunity

Discussion

Bovine mastitis is a complex trait caused by many agents, including bacteria, fungi, viruses, and physical factors. On the other hand mastitis is a polygenetic trait and is controlled by many genes. Due to the advancement in the area of molecular and genomic technology, a major increase in biological quality of data has occurred. Recently many genes have been screened by various useful bioinformatics methods such as GWAS, RNA-seq, and microarray screening, which were associated with the development or resistance of bovine mastitis. In current research by using an online bioinformatics web, we find out that the genes involved in mastitis also play a role in other diseases signaling such as leishmaniasis, Chagas disease, legionellosis, salmonellosis, toxoplasmosis regulating signaling etc.

The wealth of bioinformatics applications in the field of life sciences, including protein-protein interaction and biological pathways, has changed the paradigm of research and utilization of knowledge (Sousa et al., 2016). Interestingly, our research showed that various diseases associated signaling, including different types of cancer pathways were regulated by mastitis-associated genes (*NFKBIA*, *RELA*, *ATK1*, *MYD88* and *PIK3CA*). Several genes, such as *ATK1*, *MET*, and *PIK3CA*, were found to be involved in cancer (Scheffler et al., 2015). According to FresnoVara and his team's findings, *ATK1* interacts with the lipid kinase family and gets into the inner membrane where it has been activated by PDK1 and PDK2. The activated *ATK1* alters the functions of many substrates involved in cell survival cell cycle progression and cellular growth. That is why any alteration in the *ATK1* gene may reinforce the process of cancer (FresnoVara et al., 2004). Similarly, NF-kappa-B signaling has vital role in various human disease processes, including inflammatory infec-

tions and cancers, while *RELA* is the key member of the NF-kappa-B complex. The negative regulation of *RELA* is significantly associated with endometrial cancer (Lu and Yarbrough, 2015). These published studies verified the mastitis associated genes role in cancer. The reason might be their correlation with apoptotic processes and immune modulation, which are the main parameter for cancer development.

In the current analysis, we noticed that mastitis-associated genes also play a major role in various bacteriological (tuberculosis, salmonellosis, legionellosis), parasitic (toxoplasmosis, Chagas disease, leishmaniasis) and viral diseases (influenza A, herpes simplex infection) pathways. The possible explanation for this might be the involvement of some major processes such as apoptosis, inflammatory changes, and immune depression during mastitis, as shown in Figure 4. These are common phenomena, which can be observed in many diseases. However, the bioinformatics tools we used in the current research will be helpful to screen/predict one gene in many signaling pathways. The bioinformatics analyses revealed that the reported genes linked to mastitis have multiple roles in various diseases, which open a new gateway for future research. Although our research provides the basic information for mastitis-associated genes engagement in many biological signalings, however, it still needs practical functional validation by using animal mouse models or cell cultures.

Conclusions

The findings of this study evidenced that the mastitis-associated genes have the pleiotropic ability and play a key role in various disease pathways. Importantly, the bioinformatics tools used for evaluating mastitis-associated genes in various disease signaling provide a road map for the future research of other infectious diseases. In addition, some important mastitis-associated genes such as *NFKBIA*, *JAK2*, *STATs*, *MAPKs*, *TNF*, *RELA*, *FOS*, *JUN*, *NOS2*, *MYD88*, *TLRs*, *PIK3CA*, *NFKB1*, *IL1B* and *IL1A* were also involved in Chagas disease, influenza A, African trypanosomiasis, amoebiasis, toxoplasmosis, leishmaniasis and salmonellosis pathways; thus it would be really good to consider these genes and mentioned pathways as therapeutic signatures for the mentioned diseases. Moreover, the genes documented in this study can also be considered for future research as markers in different types of cancer.

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