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Cows selected for divergent mastitis susceptibility display a differential liver transcriptome profile after experimental *Staphylococcus aureus* mammary gland inoculation

A. Heimes,[1] J. Brodhagen,[1] R. Weikard,[1] D. Becker,[1] M. M. Meyerholz,[2,3] W. Petzl,[2] H. Zerbe,[2] H.-J. Schuberth,[3] M. Hoedemaker,[4] M. Schmicke,[5] S. Engelmann,[6,7] and C. Kühn[1,8]*

[1]Leibniz Institute for Farm Animal Biology (FBN), Institute of Genome Biology, 18196 Dummerstorf, Germany
[2]Clinic for Ruminants with Ambulatory and Herd Health Services, Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-University Munich, 85764 Oberschleißheim, Germany
[3]Immunology Unit, University of Veterinary Medicine Hannover, 30559 Hannover, Germany
[4]Clinic for Cattle, University of Veterinary Medicine Hannover, 30173 Hannover, Germany
[5]Faculty of Natural Sciences III, Martin-Luther Universität Halle-Wittenberg, 06120 Halle, Germany
[6]Technical University Braunschweig, Institute for Microbiology, 38023 Braunschweig, Germany
[7]Helmholtz Centre for Infection Research, Microbial Proteomics, 38124 Braunschweig, Germany
[8]Agricultural and Environmental Faculty, University Rostock, 18059 Rostock, Germany

ABSTRACT

Infection and inflammation of the mammary gland, and especially prevention of mastitis, are still major challenges for the dairy industry. Different approaches have been tried to reduce the incidence of mastitis. Genetic selection of cows with lower susceptibility to mastitis promises sustainable success in this regard. *Bos taurus* autosome (BTA) 18, particularly the region between 43 and 59 Mb, harbors quantitative trait loci (QTL) for somatic cell score, a surrogate trait for mastitis susceptibility. Scrutinizing the molecular bases hereof, we challenged udders from half-sib heifers having inherited either favorable paternal haplotypes for somatic cell score (Q) or unfavorable haplotypes (q) with the *Staphylococcus aureus* pathogen. RNA sequencing was used for an in-depth analysis of challenge-related alterations in the hepatic transcriptome. Liver exerts highly relevant immune functions aside from being the key metabolic organ. Hence, a holistic approach focusing on the liver enabled us to identify challenge-related and genotype-dependent differentially expressed genes and underlying regulatory networks. In response to the *S. aureus* challenge, we found that heifers with Q haplotypes displayed more activated immune genes and pathways after *S. aureus* challenge compared with their q half-sibs. Furthermore, we found a significant enrichment of differentially expressed loci in the genomic target region on BTA18, suggesting the existence of a regionally acting regulatory element with effects on a variety of genes in this region.

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*Corresponding author: kuehn@fbn-dummerstorf.de

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INTRODUCTION

Rapid progress in molecular biology and genetics has made it possible to include information on genomic variance in modern animal breeding alternatively or complementary to the classical pedigree-based approach (Meuwissen et al., 2001; Matthews et al., 2019). Facing the challenges of rising antimicrobial resistances and an increasing awareness of animal welfare issues, this approach is particularly interesting for improving animal health (e.g., in dairy cattle). Genomic selection for reduced mastitis susceptibility is a sustainable option to reduce disease incidence, but it requires profound knowledge on the underlying genetic loci modulating the trait. In a previous study, Brand et al. (2009) identified QTL on BTA18 associated with SCS (calculated from the SCC) in the German Holstein population. The SCS strongly correlates with mastitis (Weller et al., 1992; Rupp and Boichard, 2003), the infection and inflammation of the mammary gland, one of the most common infectious diseases in dairy cows (Halasa et al., 2007; De Vliegher et al., 2012). Kühn et al. (2008) described that including genetic marker haplotype information associated with the SCS QTL on BTA18 improved selection for a favorable SCS compared with selection restricted to solely conventional pedigree information. Recently, differences between alternative paternally inherited BTA18 haplotypes in clinical performance were confirmed for first lactating heifers before and after challenge with mastitis pathogens at the early lactation stage (Heimes et al., 2019; Meyerholz et al., 2019; Rohmeier et al., 2020). These

results are in line with a large number of studies reporting a major QTL for functional traits (SCS, mastitis, longevity) in the respective genomic region on BTA18 (Brand et al., 2009, 2010; Mao et al., 2016; Müller et al., 2017; Wu et al., 2017; Fang et al., 2019). Hence, information on BTA18 haplotypes in Holstein cattle breeding could contribute to improved animal health and particularly to reduced susceptibility to mastitis in the German Holstein cattle population. However, haplotype effects need to be first characterized more precisely to exclude potential detrimental side effects. Furthermore, a deep phenotypic evaluation of potential BTA18 haplotype effects could also shed light on the precise causal molecular background of the QTL localized in this chromosomal region. The genetic variation underlying this major QTL is still unclear in spite of very powerful analyses and promising candidate genes (Fang et al., 2019; Jiang et al., 2019). The liver is at the center of the metabolic and immunological physiology of the dairy cow (Moyes et al., 2016). Hence, we focused in this study on the hepatic transcriptome using RNA sequencing (**RNAseq**) to gain comprehensive information about immunological as well as metabolic differences between divergent haplotypes at the transcriptomic level. Therefore, we have looked at the effects of an intramammary challenge with a mastitis pathogen on the hepatic transcriptome of half-sib cows, which had inherited either favorable or unfavorable paternal haplotypes. The challenge was performed with *Staphylococcus aureus* strain 1027, commonly known as a causative pathogen for subclinical mastitis (Schukken et al., 2011; Jensen et al., 2013).

MATERIALS AND METHODS

Selection Process to Establish Experimental Animal Cohorts

The selection process was described in detail by Heimes et al. (2019) and Meyerholz et al. (2019). Based on previous studies (Kühn et al., 2008; Brand et al., 2009), the targeted haplotypes were allocated to 2 BTA18 sub-regions (43–48 and 53–59 Mb). As presented earlier (Meyerholz et al., 2019) for all 11,503 German Holstein AI sires born between 1999 and 2012 and recorded in the VIT genome database, we calculated SNP effects for divergent paternal haplotypes in the respective chromosomal regions (**Q**, meaning favorable for SCS, and **q**, meaning unfavorable for SCS). We selected those sires with differences of summarized SNP effects of at least 2 standard deviations larger than the mean with the assumption that those sires should be segregating for a Q or q haplotype. We excluded sires with extreme breeding values for SCS and milk performance traits to achieve similar performance levels within half-sib groups. The maternal grandsires were selected for their breeding values for SCS (Relativzuchtwert Somatischer Zellgehalt, above 112 for the Q cohort and below 100 for the q cohort). Moreover, we searched for cohorts with at least 3 potential Q and q half-sib sisters within one sire, respectively, and a maximal calving age of 36 mo. After all these filtering steps, a total of 282 heifers were genotyped with the 50k Illumina SNP chip (Illumina Inc., San Diego, CA) and haplotyped for their inherited paternal haplotypes (Q or q). Finally, 24 healthy, pregnant heifers, which originated from 6 sires, were selected. Within sire, each of the heifers was allocated to the Q or q group according to the inherited SNP haplotypes, which enabled monitoring of alternative paternally inherited haplotypes in the analysis. The selected heifers were purchased from conventional dairy farms across Germany and brought to the Clinic for Cattle at the University of Veterinary Medicine Hannover (Meyerholz et al., 2019).

Challenge Experiment

Twenty-four animals (12 Q, 12 q) were challenged in an infection model in the Clinic for Cattle at the University of Veterinary Medicine Hannover essentially as described in detail in Rohmeier et al. (2020). The husbandry of these cows was previously described by Meyerholz et al. (2019). Briefly, during the challenge experiment, the animals were kept in individual loose stall pens and received a component diet based on grass silage, corn silage, rapeseed extraction meal, soy extraction meal, concentrates, and minerals adjusted to milk performance. In the pre-challenge period, the animals were closely monitored for general health status and specific indicators of mastitis as described in Rohmeier et al. (2020). The experiment was performed under the reference number 33.12-42502-04-15/2024 with approval by the Lower Saxony Federal State Office for Consumer Protection and Food Safety. Furthermore, this study was approved by the ethics committee of the University of Veterinary Medicine Hannover. All ethical evaluations were performed as required by the German Animal Care law (Tierschutzgesetz, 2019).

The intramammary challenge experiment was based on previous work by our research group regarding selection of challenge dose and sampling time (Petzl et al., 2008, 2012). Thirty-six ± 3 d after parturition, 24 healthy animals (12 Q, 12 q) were challenged with 10,000 cfu of *S. aureus*$_{1027}$ each in both hind quarters of the mammary gland and killed 96 h later. A control udder quarter was infused with sterile sodium chloride

solution. Heifers were closely monitored for clinical signs of mastitis [e.g., declining milk yield, local (pain, swelling, redness) or systemic (fever) signs of inflammation] during the postchallenge period as described by Rohmeier et al. (2020). The animals were stunned with a penetrating captive bolt pistol, immediately followed by exsanguination via longitudinal section of the jugular veins and carotid arteries 96 h after the start of the challenge (Meyerholz et al., 2019). The time point was selected because it was predicted to be the zenith of mammary gland inflammation according to experience from previous experiments (Petzl et al., 2008, 2012). During dissection of the animals, liver tissue was collected from the *lobus caudatus*, immediately shock frozen in liquid nitrogen, and subsequently stored at −80°C. About 6 mo after sampling, the tissue was used for transcriptome analysis as described below.

Transcriptome Analysis by RNA Sequencing

Frozen liver tissue samples (approximately 30 mg) were ground using the Precellys 24 tissue homogenizer with a lysing kit containing 1.4-mm ceramic beads (peQLab, Erlangen, Germany). Total RNA was extracted via an on-column purification following the protocol of the NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany), with an adapted DNase digestion step as described by Weikard et al. (2012). The total RNA was controlled for presence of genomic DNA by PCR (Weikard et al., 2009), and a second DNase digestion step was added if required. The RNA concentration and purity were quantified on a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and a Qubit 2.0 fluorometer (Thermo Fisher Scientific), and RNA integrity was evaluated on the Bioanalyzer 2100 (Agilent Technologies, Böblingen, Germany). Subsequently, a stranded library preparation protocol for RNA sequencing was applied (TruSeq Stranded mRNA LP, Illumina) with application of indices for multiplexing during cluster generation and polyA-selection to focus on polyadenylated RNA (in the majority mRNA). The RNAseq libraries were checked for quality on the Bioanalyzer 2100. Using the Illumina HiSeq 2500 system (Illumina), we performed paired-end sequencing (2 × 90 bp).

Bioinformatic Analysis

The CASAVA v1.8 (Illumina) software was used for demultiplexing of reads. Scripts written in SAMtools (Li et al., 2009), Linux, and R (R, 2016) were applied for data processing. We checked the quality of the raw reads with FastQC version 0.11.5 (FastQC, 2016) and MultiQC version 1.4 (MultiQC, 2017). Adapters were removed using Cutadapt version 1.12 (Martin, 2011) and low-quality bases were removed using QualityTrim (Qualitytrim, 2017). The reads were aligned to the bovine reference genome UMD 3.1 with Ensembl 87 reference annotation (UMD3.1, 2016) using Hisat2 version 2.1.0 (Pertea et al., 2016). A guided transcript assembly was generated with StringTie version 1.3.2.d (Pertea et al., 2016). The advantage of this approach is that a guided transcript assembly refers to the reference genome annotation and also enables the identification of transcripts, which have not yet been annotated. Using the StringTie (Pertea et al., 2016) merge function, an annotation across samples was generated and read counting was carried out with FeatureCounts version 1.5.2 (FeatureCounts, 2017). Differential expression analysis was performed with DESeq2 version 1.18.1 (Love et al., 2014) with a threshold for significance of adjusted P (P_{adj}) < 0.05. Ingenuity Pathway Analysis (Qiagen, Hilden, Germany) was used to identify enriched biological pathways and predicted upstream regulators in response to a pathogen challenge.

During the selection process for Q and q heifers, the cholesterol deficiency (**CD**) defect was detected in the German Holstein population (Kipp et al., 2016; Menzi et al., 2016). Hence, before final heifer selection, their CD carrier status was determined by haplotype analysis (Kipp et al., 2016). In the end, 4 heterozygous CD carriers (CDC according to the World Holstein Friesian Federation, http://www.whff.info/documentation/genetictraits.php#go1) were included in the design, which were offspring of a single sire. The CDC heifers were distributed evenly across the Q and q groups. The CD carrier status was included as fixed effect in the differential expression data analysis.

RESULTS

RNA Sequencing Statistics

The transcriptome analysis by RNAseq generated 2.6 billion reads (on average 107 million reads per sample). A total of 98% of reads mapped at least once to the reference genome UMD3.1 (UMD3.1, 2016). The data analysis revealed a total of 20,723 loci, which showed an expression level of at least 10 reads in at least 4 samples.

Differential Hepatic Transcriptome Expression in Animals Infected with S. aureus

In the expression analysis comparing *S. aureus* challenged animals that had either inherited the paternal

Table 1. All significantly (adjusted *P*-value, $P_{adj} < 0.05$) differentially expressed annotated genes in the differential expression analysis between paternally inherited haplotypes Q (favorable for SCS) versus q (unfavorable for SCS) of animals challenged with *Staphylococcus aureus*

Gene symbol	Entrez gene name	Function	Log_2 fold change	P_{adj}
DYSF	Dysferlin	Regulates cell adhesion in monocytes (de Morrée et al., 2013)	0.36	0.015
MS4A3	Membrane spanning 4-domains A3	Involved in cell cycle control (Kutok et al., 2011)	4.43	0.017
GMIP	GEM interacting protein	Involved in vesicular transport and exocytosis (Johnson et al., 2012)	0.46	0.017
HMOX1	Heme oxygenase 1	Immunomodulatory and anti-inflammatory functions (Naito et al., 2014)	0.97	0.019
GPBAR1	G protein-coupled bile acid receptor 1	Involved in bile acid homeostasis and liver immunity (Biagioli et al., 2019)	0.58	0.019
MARCH5	Membrane associated ring-CH-type finger 5	Involved in the regulation of mitochondrial morphology (Tang et al., 2018)	−0.26	0.019
IGSF11	Immunoglobulin superfamily member 11	Inhibits T cell function (Wang et al., 2019a)	−0.41	0.019
FERMT2	Fermitin family member 2	Involved in TGFB1-, integrin-, Erk/MAPK-signaling pathway (Wan et al., 2015; Rognoni et al., 2016)	−0.29	0.020
ICAM3	Intercellular adhesion molecule 3	Chemotactic for macrophages, involved in phagocytosis (Torr et al., 2012)	0.53	0.022
PLCB2	Phospholipase C β 2	Inhibits expression of pro-inflammatory cytokines, regulates macrophage function (Grinberg et al., 2009; Wang et al., 2019b)	0.50	0.022
TNFAIP8L2	TNF α-induced protein 8 like 2	Negative immune regulator (Lin et al., 2018)	0.47	0.031
DNASE2	Deoxyribonuclease 2, lysosomal	Clears the cell of damaged DNA (Hacohen and Lan, 2019)	0.43	0.037
RNASE6	Ribonuclease A family member k6	Expressed by monocytes and neutrophils, antimicrobial activity (Pulido et al., 2016)	0.53	0.046
ARRDC3	Arrestin domain containing 3	Involved in placental development and pathogenesis of cancer (Lei et al., 2020)	−0.63	0.046
TNC	Tenascin C	Activator of innate immunity, upregulated as response to inflammation (Goh et al., 2010)	2.46	0.048
ZNF227	Zinc finger protein 227	Function unknown	0.29	0.048
PPP2R3A	Protein phosphatase 2 regulatory subunit B″α	Major cellular Ser/Thr protein phosphatase, known for involvement in liver cancer (Chen et al., 2019)	−0.42	0.048

haplotypes Q (n = 12) or q (n = 12), we found a total of 23 significantly ($P_{adj} < 0.05$) differentially expressed (**DE**) loci (see Table 1 and Supplemental File S1; https://doi.org/10.3168/jds.2019-17612). Of these 23 significantly DE loci, 17 were annotated genes (74%), with 12 and 5 genes expressed at a higher and lower level, respectively, in Q compared with q animals.

Within the 17 annotated DE genes between paternally inherited haplotypes Q versus q of *S. aureus* challenged animals 10 genes are listed, which are involved in immune response [e.g., *IGSF11* with known effects on human T cells (Wang et al., 2019a) and *ICAM3*, which is involved in apoptotic cell clearance (Torr et al., 2012)]. However, the top scorer of DE loci is a yet unannotated transcript on BTA18 located 2 Mb outside the genomic target region for our haplotype selection (see Supplemental File S1). Another locus localized on BTA18 at 48.9 Mb (according the UMD3.1) and still unannotated at the respective position in the current genome annotation ARS-UCD1.2 Ensembl 97 (https://www.ensembl.org/Bos_taurus/Info/Index) showed significant differential expression (see Supplemental File S1). The respective transcript does also not show any sequence homology to annotated genes in human or mouse genomes. Three DE ($P_{adj} < 0.05$) loci are localized in the genomic region spanning 43 to 59 Mb containing the targeted chromosomal subregions for haplotype selection on BTA18. Within this region, a total of 525 loci are expressed in our data set. Thus, compared with the total number of 23 DE loci across all 20,724 expressed genomic loci in the entire genome there is a significant enrichment ($P < 0.002$) of DE loci in the BTA18 genomic region 43 to 59 Mb.

Pathway Analysis of DE Genes in Animals Infected with S. aureus

Ingenuity Pathway Analysis of those 17 DE loci, which are annotated in the bovine reference genome assembly (UMD3.1), provided 24 significantly ($P < 0.05$) enriched canonical pathways when comparing *S. aureus* challenged animals that had either inher-

Table 2. All significantly ($P < 0.05$) canonical pathways enriched in the hepatic transcriptome of animals challenged with *Staphylococcus aureus* with haplotypes Q (favorable for SCS) versus q (unfavorable for SCS) using Ingenuity Pathway Analysis (Qiagen, Hilden, Germany)

Ingenuity canonical pathway	$-\log_{10}$ (P-value)
Phospholipases	2.94E00
Antioxidant action of vitamin C	2.55E00
GPCR-mediated nutrient sensing in enteroendocrine cells	2.52E00
p70S6K signaling	2.41E00
D-myo-inositol-5-phosphate metabolism	2.25E00
Gαq signaling	2.24E00
Dopamine-DARPP32 feedback in cAMP signaling	2.18E00
Heme degradation	2.12E00
ILK signaling	2.11E00
Synaptic long-term depression	2.09E00
Endothelin-1 signaling	2.07E00
IL-8 signaling	2.06E00
Breast cancer regulation by Stathmin1	2.05E00
mTOR signaling	2.01E00
Superpathway of inositol phosphate compounds	1.99E00
Phospholipase C signaling	1.84E00
Choline biosynthesis III	1.78E00
Xenobiotic metabolism signaling	1.77E00
G Protein signaling mediated by tubby	1.63E00
D-myo-inositol (1,4,5)-trisphosphate biosynthesis	1.62E00
Cell cycle regulation by BTG family proteins	1.60E00
Role of CHK proteins in cell cycle checkpoint control	1.41E00
Wnt/Ca+ pathway	1.35E00
Mitotic roles of polo-like kinase	1.35E00

ited the paternal haplotypes Q or q (see Table 2 and Supplemental File S2; https://doi.org/10.3168/jds.2019-17612). Among them are the pathways integrin-linked protein kinase (**ILK**) signaling, IL-8 signaling, phospholipases, and phospholipase C signaling that are known to be directly related to immune response. The GPCR-mediated nutrient sensing in enteroendocrine cells, which was also significantly enriched between the paternal haplotypes Q or q, provides a link between the immune response and the dairy cow's metabolism (Husted et al., 2017; Latorraca et al., 2017).

The analysis of the predicted upstream regulators (see Table 3) confirmed modulation of the hepatic immune system in Q heifers compared with the q half-sibs within *S. aureus* challenged animals. In the list of the top 10 significantly enriched upstream regulators (Table 3), 2 important immune receptors (**CXCR3**, C-X-C chemokine receptor type 3, and **TLR3**, toll-like receptor 3) as well as mitogen-activated protein kinase kinase kinase 1 (**MAP3K1**), a kinase involved in the TNF-α and NFκB signal cascades (Ishizuka et al., 1997), were found.

DISCUSSION

The heifers investigated in this study had been deeply phenotyped before and after the intramammary pathogen challenge as reported by Heimes et al. (2019), Meyerholz et al. (2019), and Rohmeier et al. (2020). The comparison of heifers with Q and q haplotypes revealed that the q animals were more susceptible to early-lactation diseases. They had a higher number of udder quarters with very low cell count (<10,000 cells/mL) in the first weeks of lactation, but in the later course of lactation the weekly SCS was significantly lower for Q compared with q animals. The q animals had a higher SCS at 24 and 36 h after *S. aureus* challenge and a higher shedding of bacteria 12 h after challenge as well as a lower decline in milk yield after pathogen challenge (Heimes et al., 2019; Meyerholz et al., 2019; Rohmeier et al., 2020). Most interestingly, in addition to differences in clinical parameters there were also significant differences in the endocrine and metabolic profiles of the animals postpartum: those cows with Q haplotypes displayed higher IGF-1, lower growth hormone, and BHB levels in plasma and serum, respectively, compared with q half-sibs (Meyerholz et al., 2019).

The hepatic transcriptome is suitable to monitor potential metabolic as well as immunological effects of the BTA18 haplotypes, as the liver is at the center of the metabolic and immunological physiology of the dairy cow (Moyes et al., 2016). To follow up the differences in IGF-1, growth hormone, and BHB plasma/serum levels between Q and q cows we had observed before the challenge, we looked at potential related hepatic transcriptomic signatures in Q versus q after intramammary *S. aureus* challenge. However, we did not find significant differences in gene expression for *IGF1* or other key genes directly involved in (short chain) fatty acid metabolism (Supplemental File S1; https://doi.org/10.3168/jds.2019-17612). Interestingly, the canonical pathway GPCR-mediated nutrient sensing in enteroendocrine cells was significantly enriched in the hepatic transcriptome of *S. aureus* challenged animals with the Q haplotype compared with animals

Table 3. Top 10 significantly ($P < 0.05$) enriched upstream regulators in Ingenuity Pathway Analysis (Qiagen, Hilden, Germany) of haplotype Q (favorable for SCS) versus q (unfavorable for SCS) within animals challenged with *Staphylococcus aureus* (exogenous chemical excluded)

Upstream regulator	P-value of overlap
GCH1	2.79E-05
SRF	1.03E-04
CXCR3	1.51E-04
KDM3A	4.10E-04
GABPA	4.51E-04
FBXL17	7.35E-04
BRIP1	7.35E-04
MAP3K1	7.66E-04
TLR3	8.89E-04
CAT	9.68E-04

with the q haplotype. G-protein-coupled receptors (**GPCR**) are membrane proteins, which detect their ligands (e.g., hormones, neurotransmitters, chemokines) in the extracellular matrix and can subsequently initiate the respective intracellular signaling cascades (Latorraca et al., 2017). In the gastrointestinal tract, nutrient metabolites activate GPCR, resulting in the secretion of gut and pancreatic hormones (Husted et al., 2017). However, GPCR can also act as pro- and anti-inflammatory regulators of immune cells (Husted et al., 2017). Thus, they have a broad spectrum of influence, and the enrichment of the respective pathway in the hepatic transcriptome of *S. aureus* challenged animals with Q haplotypes compared with the half-sibs carrying a q haplotype indicates haplotype-specific differences regarding their metabolic and immune profiles.

After the intramammary *S. aureus* challenge, the livers of Q or q cows showed a different hepatic transcriptomic profile with respect to immune genes (see Tables 1 and 2).

The enrichment of the canonical pathways ILK signaling and IL-8 signaling indicates that immune pathways play a dominant role in the response of the divergent hepatic transcriptome response to intramammary *S. aureus* challenge. This is also indicated by the nominally significant enrichment of the Protein Information Resource category "Innate immunity" ($P = 1.1 \times 10^{-3}$) and also by the significantly DE genes themselves (see Table 1). DYSF, for example, which was originally observed for its role in muscle function, is involved in regulating cellular interactions, cell adhesion mechanisms, and has a function in inflammatory cells (de Morrée et al., 2013). The receptor encoded by *GPBAR1* is implicated in the suppression of macrophage functions and regulation of energy homeostasis by bile acids and is also essential for the regulation of liver immunity (Biagioli et al., 2019). HMOX1 has been recognized as having major immunomodulatory and anti-inflammatory properties and has been regarded as an adaptive cellular response against inflammatory response and oxidative stress (reviewed by Naito et al., 2014). FERMT2 plays a role in the TGFB1 and integrin signaling pathways (reviewed by Rognoni et al., 2016) and is related to the Erk/MAPK signaling pathway (Wan et al., 2015). Furthermore, *ICAM3* and *IGSF11* are both members of the immunoglobulin super-family. ICAM3 has been described as chemotactic for macrophages and is therefore implicated in the phagocytosis of apoptotic cells (Torr et al., 2012), whereas IGSF11 inhibited T cell function in human blood cell culture (Wang et al., 2019a). TNC, described to be upregulated in inflamed tissues, is an activator of innate immunity, which can stimulate the synthesis of inflammatory cytokines (Goh et al., 2010). The *PLCB2* gene, also significantly differentially expressed, is part of the significantly enriched canonical pathways phospholipases and phospholipase C signaling. As a key regulator for macrophage function, PLCB2 is involved in the switch from an inflammatory (M1) to an angiogenic (M2-like) macrophage phenotype (Grinberg et al., 2009). Moreover, it can inhibit the expression of pro-inflammatory cytokines (Wang et al., 2019b). As presented in Figure 1, the Ingenuity network analysis showed that *DYSF*, *GPBAR1*, *HMOX1*, *FERMT2*, *ICAM3*, *TNC*, and *PLCB2* are interacting with each other in a network related to immune response, indicating an interrelationship between them.

As potential transcriptional regulators CXCR3, TLR3, and MAP3K1 could be predicted (Table 3). The CXCR3 is expressed by monocytes, T cells, natural killer cells, dendritic cells, and cancer cells (Tokunaga et al., 2018). After activation by its selective ligands CXCL9, CXCL10, and CXCL11, it is involved in the recruitment and clustering of T cells and natural killer cells (Tokunaga et al., 2018; Maurice et al., 2019; Read et al., 2019). Toll-like receptor 3 is widely known for its crucial role in pathogen recognition and subsequent activation of innate immunity (Chen et al., 2017). Mitogen-activated protein kinase kinase kinase 1 takes part in the TNF-α and NFκB signal cascades (Ishizuka et al., 1997; Sanchez-Perez et al., 2002) and is therefore eminent for the appropriate response of the immune system to invasive pathogens. Thus, the upstream analysis confirmed alteration of the hepatic immune system in Q heifers compared with their q half-sibs within *S. aureus*-challenged animals.

Regarding a potential molecular background of the QTL for functional traits and udder health in particular, we found a significant enrichment of DE loci in the genomic target area of the different haplotypes and their close vicinity on BTA18. This suggests that a regionally acting regulatory element might be involved, which could modulate the expression of an array of genes located there. Regulatory variation in noncoding regions as background of the BTA18 telomeric functional QTL might also explain the lack of causal candidate variant evidence for this QTL. No causal mechanism for the QTL could be pinpointed despite genetic variants with predicted effects in coding and noncoding regions, as described in recent GWAS at the whole genome sequence level with powerful data sets (Wang et al., 2017; Fang et al., 2019; Jiang et al., 2019). This also underlines the need to improve the current annotation of the bovine genome in terms of functional regula-

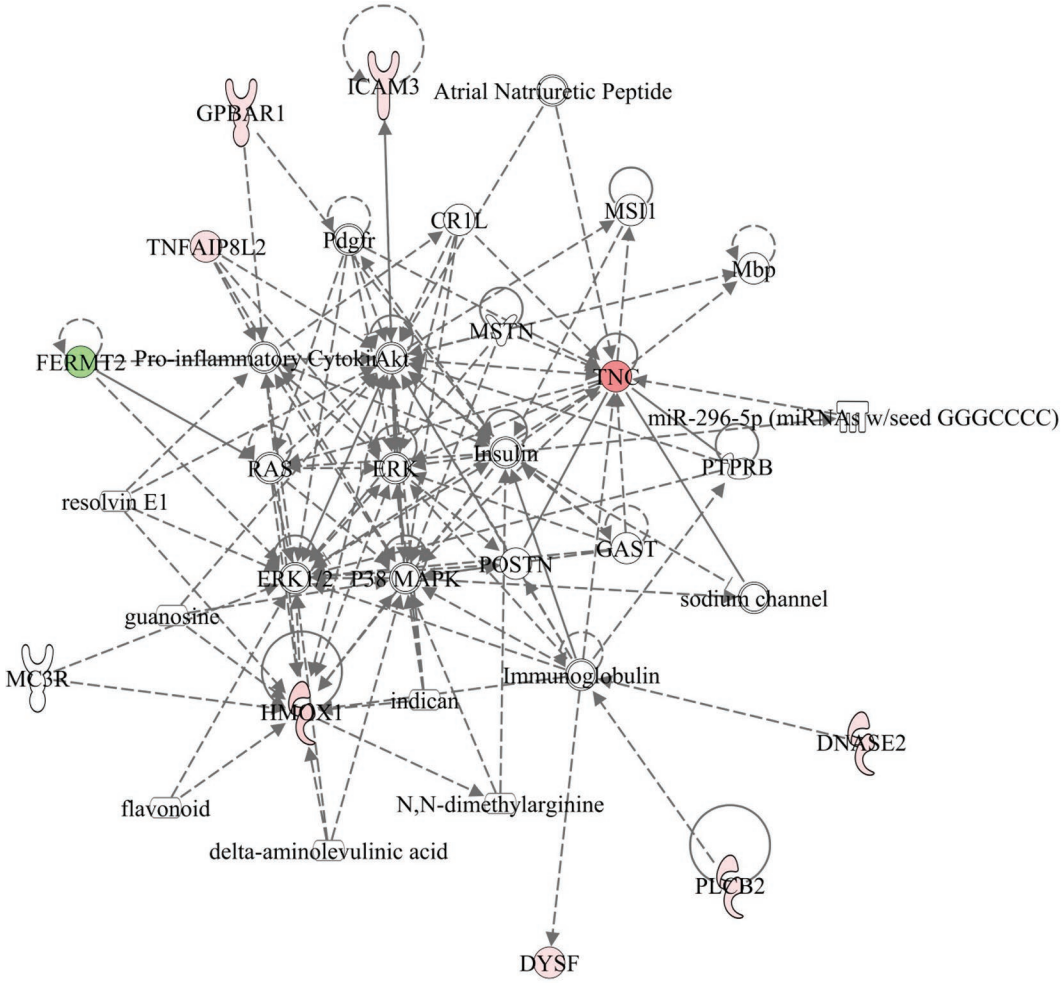

Figure 1. Highly enriched network in the differentially expressed gene analysis of Q (favorable paternal haplotypes for SCS) versus q (unfavorable haplotypes for SCS) in cows challenged with *Staphylococcus aureus*. Green = lower expression in Q cows compared with q cows, red = higher expression in Q compared with q cows (modified according to Ingenuity Pathway Analysis, Qiagen, Hilden, Germany).

tory elements as addressed by the global Functional Annotation of Animal Genomes action (https://www.animalgenome.org/community/FAANG/; Andersson et al., 2015).

CONCLUSIONS

Heifers with alternative paternal BTA18 haplotypes display a significantly different hepatic transcriptome upon intramammary *S. aureus* challenge. Animals with the favorable Q haplotypes showed a more activated immune system (as demonstrated by the higher activation of key pathways and divergent expression of relevant immune genes), which might be better able to defend the host during a bacterial challenge. Driver of these differences in the immune system might be different adaptations of metabolic pathways of the Q and q animals. These findings are in line with results from clinical data of the haplotypes, which showed Q cows to be more resistant to diseases, especially *S. aureus* mastitis (SCC, SCS, shedding of bacteria, milk yield) than their q half-sibs.

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ORCIDS

A. Heimes https://orcid.org/0000-0003-0638-0596
R. Weikard https://orcid.org/0000-0002-0308-9966
D. Becker https://orcid.org/0000-0003-0425-6284
M. M. Meyerholz https://orcid.org/0000-0003-1720-6593
W. Petzl https://orcid.org/0000-0002-7931-2642
H. Zerbe https://orcid.org/0000-0002-7972-4858
H.-J. Schuberth https://orcid.org/0000-0002-5904-5751
M. Hoedemaker https://orcid.org/0000-0001-8154-2642
M. Schmicke https://orcid.org/0000-0001-7218-293X
C. Kühn https://orcid.org/0000-0002-0216-424X