ChronMast - a model to study functional genetic variation of mastitis susceptibility

M. Meyerholz[1,2], A. Heimes[3], J. Brodhagen[3], L. Rohmeier[2], T. Eickhoff[1], S. Jander[1], A. Hülsebusch[1], R. Weikard[3], J. Günther[3], H.-M. Seyfert[3], H. Zerbe[2], W. Petzl[2], M. Hoedemaker[4], H.-J. Schuberth[1], S. Engelmann[5,6] & Ch. Kühn[3,7]

[1] *Immunology Unit, University of Veterinary Medicine Hannover, 30173 Hannover, Germany*
[2] *Clinic for Ruminants, Ludwig-Maximilians-University Munich, 85764 Oberschleißheim, Germany*
[3] *Leibniz Institute for Farm Animal Biology, Genome Biology, 18196 Dummerstorf, Germany*
[4] *Clinic for Cattle, University of Veterinary Medicine Hannover, 30173 Hannover, Germany*
[5] *Institute for Microbiology, Technical University Braunschweig, 38124 Braunschweig, Germany*
[6] *Microbial Proteomics, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany*
[7] *Agricultural and Environmental Faculty, University Rostock, 18059 Rostock, Germany*
kuehn@fbn-dummerstorf.de (Corresponding Author)

Summary

Biologically informed breeding requires detailed knowledge on the genomic variants modulating the target phenotype as well as on the potential (patho)physiological consequences of those variants. In dairy cattle, mastitis is one of the most frequent and costly diseases. However, there is limited progress in elucidating the genomic and physiological background of its genetic variation. Based on a previously established animal model and on further data from the literature, we set up an experimental design with half sib groups of heifers inheriting alternative paternal haplotypes on a target interval on *Bos taurus* chromosome 18 (BTA18). Sires were selected for highly divergent SNP effects of the alternative haplotypes on somatic cell score, an indicator trait for mastitis incidence, and heifers were assigned to experimental groups according to their inheritance of either the favourable (GQ) or unfavourable (kq) paternal haplotype. The animals were allocated six weeks prior to first calving as either long-term cell donors (LT) until day 42 of the second lactation (n=6) or for experimental challenge with mastitis pathogens (*E. coli, S. aureus*) (IC) at day 36 ± 3.4 of the first lactation (n=36). Significantly more kq animals compared to GQ individuals were diagnosed with one or more infectious diseases (P<0.05) between parturition and the start of the mastitis challenge at about five weeks after calving. Furthermore, during the course of lactation the kq animals of the long-term group displayed significantly higher somatic cell score compared to GQ. Interestingly, in the first six weeks after calving the proportion of quarters with extremely low somatic cell count (< 10,000 cells/ml) was significantly higher in both kq groups (LT, IC) compared to their GQ counterparts (P < 0.01, P < 0.05, respectively). The differences in disease incidence and in somatic cell score particularly during the second stage of lactation indicate that indeed the groups GQ and kq comprise animals of divergent susceptibility for infectious diseases. The mastitis challenge experiment and comprehensive subsequent immunological, endocrinological,

immunohistochemical and transcriptomic analyses will reveal potential differences concerning the response towards the intramammary infection. These differences will provide knowledge on the (patho)physiological background of divergent mastitis susceptibility and will aid the detection of the underlying genetic variation.

Keywords: mastitis, somatic cell count, challenge, animal model

Introduction

Due to recent advances in high-throughput genotyping and whole genome sequencing, substantial progress has been achieved in fine-mapping and even identification of genetic variation modulating quantitative traits (Pausch *et al.* 2017). However, particularly for traits of low heritability, these approaches often come to their limits.

Selection gain can be very effectively increased by genomic selection (Schaeffer 2006). To avoid negative consequences, however, it would be beneficial to know in advance, which particular physiological mechanisms will be preferentially addressed to avoid negative consequences of selection strategies. Biologically informed breeding requires an improved knowledge of the pathways and regulatory networks, which are the major physiological contributors to the genetically determined phenotypic variation. In addition, it will add functional information to complement the high-resolution fine-mapping data for improved identification of causal genetic variants.

Mastitis is one of the most frequent and costly diseases in dairy cattle (Halasa *et al.* 2007). Whereas multiple studies have reported the presence of genetic loci underlying genetic variation of this trait (https://www.animalgenome.org/cgi-bin/QTLdb/BT/index), at present insufficient information on their specific genetic and (patho)physiological background has been obtained.

To address this issue, we set up an animal model of genetically determined variation for mastitis susceptibility and reproductive fitness based on previous studies reporting a genomic region on *Bos taurus* chromosome 18 (BTA18) associated with somatic cell score (SCS), mastitis and calving difficulty (Kuehn *et al.* 2003; Brand *et al.* 2009; Brand *et al.* 2010; Cole *et al.* 2011; Fang *et al.* 2017; Wang *et al.* 2017).

Set-up of the model

Selection of heifers with presumably divergent susceptibility for mastitis

Haplotyping was performed for all individuals using genotyping data within the VIT genome data base for German Holsteins (Segelke *et al.* 2014). The male haplotyped individuals were filtered for date of birth (later than 1999 and before 2012) and breed registration status of sire for German Holsteins, which left a total of 11,503 sires to for further selection with a high probability to have heifers at the beginning of the experimental period. Somatic cell count is a indicator for mastitis incidence and thus served as target phenotype. Estimation of SNP allele effects was performed within the routine genomic evaluation for somatic cell count for German Holstein applying a two-step marker BLUP approach (www.vit.de).

From the evaluation run in August 2015, SNP effects were summarised for BTA18 haplotypes and for two BTA18 sub-regions (43 - 48 Mb and 53 - 59 Mb). The margins of the sub-regions were determined from a previous BTA18 mastitis model (Mas.net), which had been set up according to microsatellite genotyping data (Kuhn 2008; Brand *et al.* 2011): the

BTA18 50k SNP haplotypes from the three sires of the previously established Mas.net half-sib ships were obtained from the initial haplotyping run (see above). The 50k SNP genotypes of the Mas.net heifers were used to assign the favourable (GQ) or unfavourable (kq) status to the sires' haplotypes. Manual overlap identified three regions common to the three kq haplotypes of the three Mas.net sires involved. These three regions in addition to data from the literature (Brand *et al.* 2009; Cole *et al.* 2009; Cole *et al.* 2011) demarcated the margins of the selected genomic regions for this project.

For the regions, differences between SNP effects for alternative paternal haplotypes for all 11,503 sire selection candidates were established. Sires were selected, which displayed differences of summarized SNP effects of at least two standard deviations larger than the mean haplotype difference of all sires for the interval 43–65 Mb. Furthermore, we required the difference in at least one of the subregions 43-48 or 53-59 Mb to be larger than two standard deviations from the mean. Finally, the sires should not have inverse phasing regarding the direction of the haplotype differences between those two subintervals. This filtering step left 156 sires to be considered for further heifer selection steps. The female offspring of those 156 sires were filtered for age at the beginning of the experiments (at least 18 months of age) and anticipated day of calving (from insemination records to select heifers calving within the experimental time window).

Sires with extreme breeding values for somatic cell score and milk performance traits were excluded, because we aimed at high variability within half sib group, but similar performance level between half sib groups. Thus, we considered only heifers from sires with a normalized breeding value for milk performance (RZM)of at least 100, a normalized breeding value for somatic cell count (RZS) of 88–112 (for daughter proven sires) or 94–118 (for sires with exclusively genomic information), and an RZD (an index for milk flow and milkability) of 88–112. Furthermore, we asked for the dam sires' RZS to be either below 100 (for the kq heifer cohort) or above 112 (for the GQ cohort). The list of heifers obtained after the filtering steps was then screened for sib ships with more than three potential GQ and three potential kq individuals and maximal age at calving (36 months). A final 282 (1st selection period: 140, 2nd selection period 142) heifers, were genotyped with the 50k Illumina SNP chip, haplotyped and allocated to the paternal haplotype (either GQ or kq), which they had inherited. During the selection process, the genetic defect cholesterol deficiency (CD) was discovered (Kipp *et al.* 2016), and we were careful to select an equal proportion of CD carriers for the GQ and kq group to avoid potential bias of the results due to differences in lipid metabolism of the selection candidates. In addition to balancing for CD status (4 CD carriers in the GQ and 3 CD carriers in the kq group), GQ and kq groups were also balanced for sire by creating halfsib groups of similar numbers within GQ and kq. In total, both groups GQ and kq, comprised offspring of the same six sires (Figure 1).

Those heifers meeting all health and veterinary requirements were purchased and allocated about six weeks prior to first calving either to the animal experimental unit of the FBN Dummerstorf (n=6, 3 GQ, 3 kq) for a long-term model (LT) or to the Clinic for Cattle at the Veterinary University of Hanover (TiHo) (n=36, 18 GQ, 18 kq) for an infection challenge model (IC).
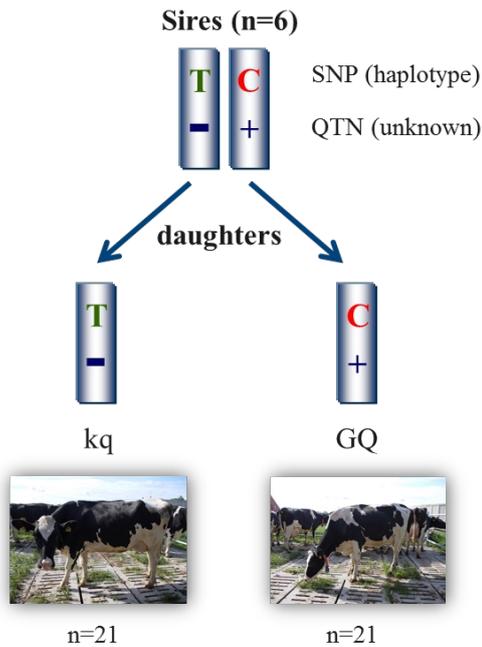
Figure 1: Set-up of the animal model for divergent susceptibility to mastitis

Animal management and clinical investigations

At the FBN Dummerstorf, heifers were kept in a loose stall barn and fed a total mixed ration with 6.5 MJ NEL prior calving and 7.0 MJ NEL/kg DM after calving. Heifers were milked in a tandem milking parlor twice a day with daily milk volume measurement. In addition, daily feed intake was measured (Roughage Intake Control, Insentec, Marknesse, The Netherlands). At the Clinic for Cattle in Hanover, the heifers were kept in individual loose stall pens and fed a component ratio comprising concentrates (2-8 kg per day according to individual milk yield), grass silage, corn silage, minerals, canola and soybean extraction meal as well as hay and were milked twice a day. After an adaptation period of one week, all LT and IC heifers were monitored for comprehensive clinical parameters and body weight prior and after calving. In addition, daily feed intake was recorded. Weekly quarter milk samples were collected for analysis of milk components and somatic cell score.

After calving, all IC animals at the Veterinary University of Hannover received a subcutaneous treatment with enrofloxacin for five days (2.5 g, Enrotron 10%®, aniMedica GmbH, Germany) to erase any pre-existing microbial infection. The heifers were challenged then with mastitis pathogens 36 ± 3.4 days after calving and slaughtered 24 h (*E. coli* challenge; n=12, 6 GQ, 6 kq) or 96 h (*S. aureus* challenge; n=24, 12 GQ, 12 kq) after challenge. The heifers at the FBN completed their first lactation and were slaughtered 42 days after second parturition.

Statistical analyses

Potential differences between GQ and kq heifers regarding disease incidence and number of udder quarters with extremely low somatic cell score were evaluated by chi square test or Fisher exact test, when the subgroups were smaller than five animals. From the daily raw milk volume data, average daily milk volume was calculated for each week. Daily energy corrected milk yield (ECM) was calculated according to Kirchgessner (1997). For the

evaluation of potential differences between groups for ECM and somatic cell score ($\log_2$(somatic cell counts per ml/100,000) + 3), a linear model was applied fitting the effects of week and group and their interaction as fixed effects.

Phenotypic clinical investigation of the animal model for genetically divergent susceptibility for mastitis

Prior to calving, the kq and GQ heifers did not differ regarding disease incidence. However, after parturition, within the TiHo heifers, significantly more kq animals compared to GQ individuals were diagnosed with one or more infectious diseases (P<0.05) between parturition and the start of the mastitis challenge at about five weeks after calving. Analogous numerical observations were obtained for the FBN heifers (three infectious diseases in the GQ group compared to six in the kq group). Within the infectious diseases, the dominant feature was metritis, which was significantly more frequent in the kq heifers compared to the GQ heifers (P < 0.05). The average time for metritis treatment did not differ between groups.

In week 5 after parturition, kq heifers displayed significantly lower somatic cell score compared to GQ animals (P <0.05) (Figure 2). In week 4 and 6, those differences showed tentative significance. We observed analogous results for the FBN heifers: kq animals had a significantly lower somatic cell count in week 3-4 (P<0.05). However, across the later course of the first lactation, the kq animals had a higher somatic cell count compared to GQ (P < 0.001, Figure 3). The average energy corrected milk for the first lactation of the FBN group was higher for the GQ than for the kq heifers (P<0.001) (Figure 3).
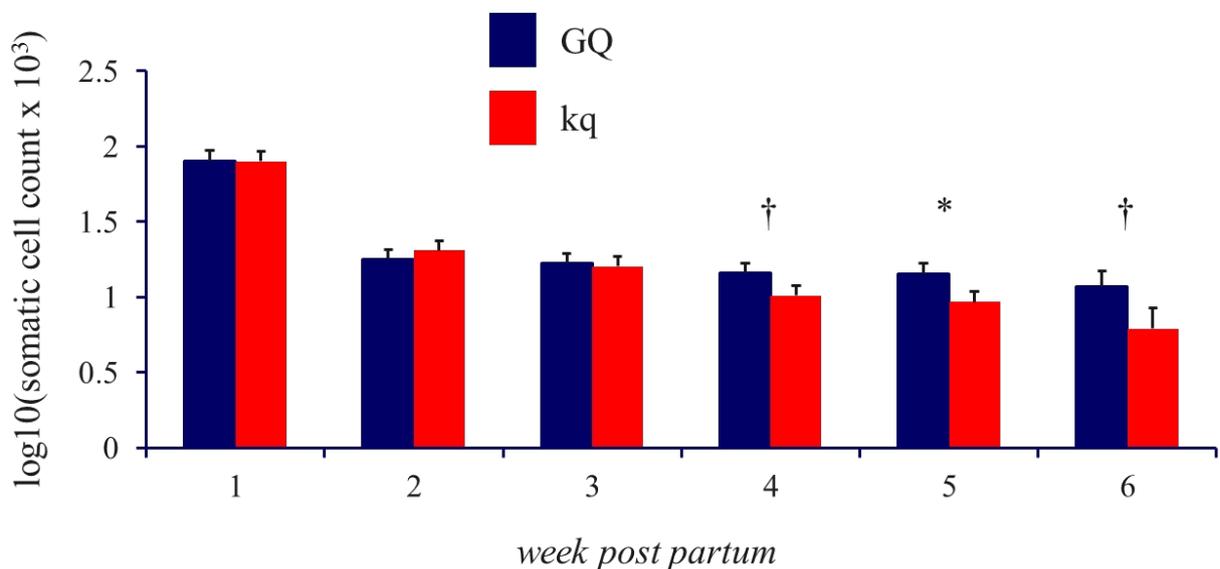

*Figure 2: Somatic cell count in whole milking from TiHo GQ and kq heifers after calving. *: P < 0.05, †: P < 0.10.*
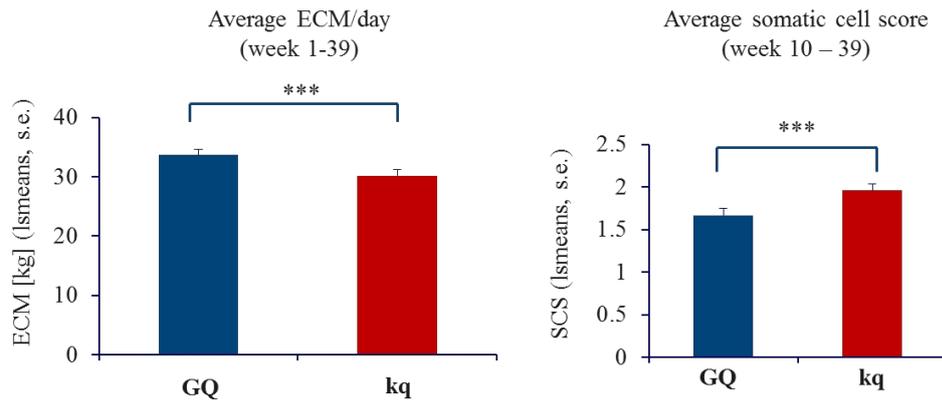
Figure 3: Average energy-corrected daily milk yield and average somatic cell score in the first lactation of FBN GQ and kq heifers. ***: P < 0.001.

When looking at individual quarter milk samples of the TiHo animals, the proportion of quarters with extremely low somatic cell count (< 10,000 cells/ml) was significantly (P < 0.01) higher in the kq compared to GQ group (Figure 4). Analogous results were obtained for the FBN animals.
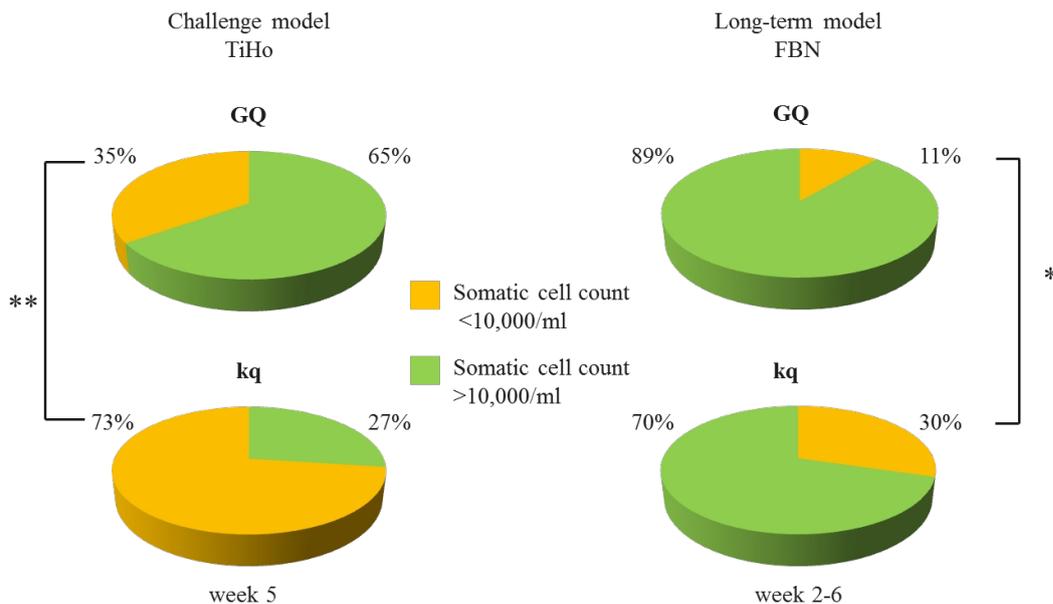

Figure 4: Proportion of udder quarters with a somatic cell count below and above 10,000 somatic cells/ml milk after parturition in the TiHo and FBN GQ and kq groups. **: P < 0.01

Evaluation of the mastitis susceptibility model

Clinical data obtained during the first five weeks after calving revealed significant differences in the susceptibility to infectious diseases between the two groups, which had been selected for their BTA18 sub-haplotype SNP effects for somatic cell score. This indicates that indeed

the two groups should differ in their potential to fight infectious diseases. The dominant feature in kq heifers was an elevated incidence of metritis, which fits previous reports about genetic loci affecting reproductive performance in the targeted chromosomal region (Kuehn *et al.* 2003; Wang *et al.* 2017). The significantly lower SCS of kq heifers at the beginning of the lactation seems to be contradictory to the initial hypothesis of kq animals being more susceptible to mastitis. However, a significantly higher proportion of udder quarter samples with extremely low cell count (<10,000 cells/ml) at the beginning of the lactation and an elevated SCS in later lactation suggest that a minimum number of resident protective cells in the milk is essential for an appropriate response to mammary infection. Similar observations have been described previously (Suriyasathaporn *et al.* 2000).

The established model for differential disease susceptibility is an excellent model for further analysis of the response to an intramammary challenge with mastitis pathogens and is being used for experimental *E. coli* and *S. aureus* infections, according to the challenge model (Petzl *et al.* 2008). Immunological, endocrinological, immunohistochemical and transcriptomic analyses are being conducted to reveal potential differences in the response to intramammary infection. These differences will provide knowledge on the (patho)physiological background of divergent mastitis susceptibility and will aid the detection of the underlying genetic variation.

Acknowledgements

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