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The genetic background of clinical mastitis in Holstein-Friesian cattle

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Introduction

Mastitis is an inflammatory disease of the mammary gland, which has a significant economic impact and is an animal welfare concern. This work examined the association between single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) with the incidence of clinical mastitis (CM). Using information from 16 half-sib pairs of Holstein-Friesian cows (32 animals in total) we searched for genomic regions that differed between a healthy (no incidence of CM) and a mastitis-prone (multiple incidences of CM) half-sib. Three cows with average sequence depth of coverage below 10 were excluded, which left 13 half-sib pairs available for comparisons. In total, 191 CNV regions were identified, which were deleted in a mastitis-prone cow, but present in its healthy half-sib and overlapped in at least nine half-sib pairs. These regions overlapped with exons of 46 genes, among which APP (BTA1), FOXL2 (BTA1), SSFA2 (BTA2), OTUD3 (BTA2), ADORA2A (BTA17), TXNRD2 (BTA17) and NDUFS6 (BTA20) have been reported to influence CM. Moreover, two duplicated CNV regions present in nine healthy individuals and absent in their mastitis-affected half- sibs overlapped with exons of a cholinergic receptor nicotinic α 10 subunit on BTA15 and a novel gene (ENSBTAG00000008519) on BTA27. One CNV region deleted in nine mastitis-affected sibs overlapped with two neighbouring long non-coding RNA sequences located on BTA12. Single nucleotide polymorphisms with differential genotypes between a healthy and a mastitis- affected sib included 17 polymorphisms with alternate alleles in eight affected and healthy half-sib families. Three of these SNPs were located introns of genes: MET (BTA04), RNF122 (BTA27) and WRN (BTA27). In summary, structural polymorphisms in form of CNVs, putatively play a role in susceptibility to CM. Specifically, sequence deletions have a greater effect on reducing resistance against mastitis, than sequence duplications have on increasing resistance against the disease.

Keywords: copy number variation, genomic annotation, single nucleotide polymorphism, somatic mutations, whole genome sequence

Implications

We compared whole genome DNA sequence of 13 half-sib pairs, discordant for their clinical mastitis (CM) status, but matched by age, age of calving and lactation season. Results indicated that deletions of genomic regions were more likely to be associated with increased susceptibility to CM than duplications of genomic regions and single nucleotide poly- morphisms (SNPs). In total, 191 genomic regions deleted in a mastitis-prone sib and present in a healthy sib, observed in at least nine pairs, overlapped with exons of 46 genes with functions related to immune response and gene expression. Such regions can be used in selection for mastitis resistant cows.

Introduction

Mastitis is an inflammatory disease of the mammary gland that occurs in response to a physical injury or infection by pathogenic microorganisms, such as Escherichia coli, Streptococcus uberis and Staphylococcus aureus (Schukken et al., 2011). Following infection of the mammary gland, macrophages and epithelial cells release cytokines that cause the migration of neutrophils, monocytes and other leukocytes from the blood to the site of infection in the mammary tissue. The cost associated with mastitis in Eur- ope, according to current estimates, is 1.55 billion € per year (European Union http://www.sabre-eu.eu/). The frequency and cost of mastitis, and rising

public concerns for animal welfare, have made mastitis one of the most important diseases for the dairy sector (Thompson-Crispi et al., 2014).

There have been 292 genetic associations reported for risk of CM distributed across most chromosomes (Animal QTLdb www.animalgenome.org/www.animalgenome.org/QTLdb rel. 36. August 2018; Detilleux, 2009). Recent develop- ments in genome sequencing technologies applied to live- stock have facilitated the identification of copy number variations (CNVs) and millions of SNPs in a relatively cost efficient manner. This has enabled researchers to describe the genomic landscape of livestock species (e.g. Choi et al., 2014; Szyda et al., 2015; Mielczarek et al., 2017), and to combine this whole genome sequence data with phenotypic information for genomic prediction (VanRaden et al., 2017) or in genome-wide association studies to identify variations associated with various traits (Sanchez et al., 2016; Sanchez et al., 2017). Combining SNP microarray genotyping with whole genome DNA and RNA sequencing is beginning to disentangle the genomic regulation of economically impor- tant traits, such as mastitis susceptibility in dairy cattle (Fang et al., 2017).

The current work examined the association between single base pair (SNPs) and structural polymorphisms (CNVs), identi- fied in whole genome DNA sequences, with the incidence of CM. Unlike previous studies, we compared SNP and CNV var- iation identified in sequence data from 16 paternal half-sibs that were selected to be discordant for susceptibility to CM.

Material and methods

Animals

Whole genome DNA sequences were produced for 32 cows of the Holstein-Friesian breed that were selected from a dataset of 991 individuals, which had veterinary records for incidence of CM. All the cows were kept in the same barn, managed in the same way, cared for by the same staff, fed the same balanced diet in the form of a total mixed ration, and milked in the same herringbone milking parlour. A potential case of CM was reported to the stud veterinarian by the milking staff. Then a veterinarian made the definitive diagnosis based on the following clinical symptoms: (i) alterations in consistency, colour and smell of milk; (ii) occurrence of redness, swelling, pain, tissue hardening, increased temperature of udder; (iii) deterioration of a cow's general condition manifested by fever, reduced feed intake, significant decrease in milk production and anxiety. In unresolved cases microbiological tests were performed. The 32 cows comprising 16 paternal half-sibs were matched for: (i) age – half-sibs born in the same year and season (the mean age difference between half-sib pairs was 15.36 days), (ii) age at calving, and (iii) year and season of the start of lactation (mean difference ±30.19 days), but discordant in their mastitis status. Mastitis resistant cows had never been diagnosed with mastitis and had been in the herd for a minimum of three full lactations. Mastitis-prone cows had several episodes of CM in the first two full lactations. The incidence of the disease in the study group ranged between 6 and 14 cases, with a mean of 8.00. Cows with mastitis were generally culled, therefore none of those remaining in the herd to the third and subsequent lactations had the highest incidence of mastitis.

Whole genome DNA sequence and bioinformatic pipelines Whole genome sequences of the 32 cows were obtained using the Illumina HiSeq2000 Next Generation Sequencing platform. A detailed description of the protocol has been described previously (Szyda et al., 2015). The total number of raw reads generated for a single animal ranged between 164 984 147 and 472 265 620. Raw reads were aligned to the UMD3.1 reference genome using BWA-MEM software (Li and Durbin, 2009) with the number of aligned reads varying from 155 202 885 (94.07% of UMD3.1) to 454 412 859 (99.57% of UMD3.1) per animal and the average depth of coverage between 5 × and 17 × (Table 1). Resulting BAM files were processed using a combination of tools from the Picard (http://broadinstitute.github.io/picard/) and SAMtools (Li et al., 2009) packages and included re-alignment to the reference sequence as well as removal of PCR duplicates. In order to make use of the half-sib structure of the data set, the variants (SNPs and CNVs) were identified by comparison of healthy and mastitis-prone siblings. In particular, SNPs were identified by comparing the discordant sib pairs using the somatic mutation caller Varscan2 (Koboldt et al., 2012). 'Somatic' SNPs were called when a healthy cow was homozygous for a reference allele and the mastitis-prone half-sib was heterozygous or homozygous for the alternative allele. Differences in the number of reads were tested using the Fisher's Exact Test with Bonferroni multiple testing correction. The number of 'somatic' SNPs per half-sib pair ranged from 314564 to 6765038 (x=4932444±1615483) (Sup- plementary figure S1). Copy number variations were identified using the CNVnator software (Abyzov et al., 2011). Copy number variations longer than 1 Mbp were removed. To exclude false positive variants being a consequence of artefacts arising from errors in the reference genome, deletions shared by at least 15 cows were removed if they had a 50% or higher overlap with gaps in the reference genome. As CNVnator is based on differences in read depth along the genome, three cows with average depth of coverage below 10 and their respective half-sibs were excluded leaving

13 half-sib pairs which were used to identify CVNs. The total number of CNVs identified for the 26 individuals ranged between 1694 and 5187 (x = 4 932 444 ± 1 615 483) for duplicated sequences and between 9731 and 18 479 (x = 12 787 ± 1 792) for deleted sequences with lengths ranging between 200 to 724 000 bp for deletions and 200 to 439 300 bp for duplica- tions (Supplementary Figure S2). Deleted sequences covered from 2.71% to 6.09% of the whole genome, while duplications accounted for a 0.51% to 1.58% increase in the total sequence length.

Table 1 *Average depth of coverage and per-read quality score for each of the Polish Holstein-Friesian cows half-sib pairs*

Mastitis prone-sibling		Healthy sibling	
Average coverage	Average quality	Average coverage	Average quality
12 ± 53	36.23	17 ± 84	35.00
16 ± 61	34.84	15 ± 62	35.86
10 ± 42	36.64	14 ± 66	35.92
9 ± 37	*36.77*	*17 ± 79*	*34.40*
8 ± 30	*36.76*	*16 ± 73*	*35.32*
13 ± 66	35.90	16 ± 73	35.31
11 ± 61	35.63	17 ± 83	35.06
10 ± 58	36.35	16 ± 78	35.02
17 ± 70	35.13	15 ± 68	35.69
11 ± 61	36.29	14 ± 88	35.53
14 ± 64	35.68	14 ± 76	30.96
16 ± 66	35.36	17 ± 11	33.16
16 ± 74	35.08	15 ± 87	30.43
15 ± 64	35.51	16 ± 84	31.90
16 ± 66	34.95	15 ± 94	30.79
16 ± 72	*34.79*	*5 ± 35*	*25.91*

Half-sibs-pairs removed from the analysis due to a low coverage are shown in italics.

Quantifying the association of variants with clinical mastitis Somatic SNPs were identified by comparing the number of reads supporting an alternative and a reference allele between a healthy and a mastitis-prone half-sib. The 'tumour purity' parameter was set to 0.75 to reflect the genotypic similarity between half-sibs. We focused on CNVs, which were deleted in a mastitis prone cow, but present in its healthy sibling, and on CNVs that were duplicated in a healthy cow, but not duplicated in its mastitis-prone sibling. Such genomic sequences were identified by comparing CNVs in each discordant sib-pair and defined as CNV regions (CNVRs).

Annotation

The genomic annotation of SNPs and CNVs exhibiting a dif- ferential polymorphism between a mastitis-prone and a healthy sib was performed using Variant Effect Predictor software (McLaren et al., 2010). Functional, non-coding sequences were annotated based on the miRBase database (http://www.mirbase.org) for micro-RNA and the Noncode database (http://www.noncode.org), Billerey et al. (2014) and Koufariotis et al. (2015) for long non-coding RNA.

Results

Association of somatic single nucleotide polymorphisms with clinical mastitis

Altogether 672 272 SNPs were identified that showed allele differences between discordant sibs in the number of reads supporting an alternative and a reference allele, which were significant at the 10% type I error level. Among these only 17 SNPs were in common in at least eight half-sib families and were considered in further analysis. Three of these SNPs were located within introns of genes. The most common somatic SNP was found in 10 half-sib families and was located at the position 28 810 758 bp on BTA27 in an intron of RNF122, a RefSeq sequence corresponding to the gene coding for ring finger protein 122. Interestingly, ten other somatic SNPs located within this gene with alternate alleles were identified in seven or eight half-sib pairs. RNF22 encodes a protein involved in basic processes such as protein–protein and protein–DNA interactions. It may therefore be expected to play a

role in cell viability, and has been reported to have a role in immune response (Wang et al., 2016). The second most common SNP, present in nine families, was identified on BTA04 at 51 991 924 bp and overlapped with an intron of the proto-oncogene, receptor tyrosine kinase (MET, ENSBTAG00000006161), which plays a role in cellular migration and invasion, processes which are related to inflammation. The third SNP, which was common in eight families, was also located on BTA27 (26 370 233 bp) within the WRN gene (ENSBTAG00000021592), which is respon- sible for the Werner syndrome and encodes Werner syn- drome RecQ-like helicase protein. Mutations in this gene result in premature aging in humans and an increased sus- ceptibility to infection (Domínguez-Gerpe and Araújo-Vilar, 2008).

Association of copy number variant regions with clinical mastitis

The structural variation examined were (i) genome duplica- tions present predominantly in healthy animals in at least nine half-sib pairs, and (ii) genome deletions present pre- dominantly in mastitis-prone animals, also in at least nine half-sib pairs. The biological hypothesis underlying strategy (i) was that healthy sibs may harbour a duplication in a region functionally important for CM resistance, which may then be associated with a higher level of expression, while in strategy (ii) deletion of functionally important genomic region may increase the risk of CM. Altogether, 28 755 duplicated CNVRs were present in the mastitis-prone sibs and absent from the healthy sibs, among these only 36 (0.13%) CNVRs were present in at least nine sib-pairs. Two of the regions overlapped with exons of a gene coding for a cholinergic receptor nicotinic alpha 10 subunit on BTA15 and a novel Bos taurus gene with Ensembl ID ENSBTAG00000008519 located on BTA27. A more interest- ing picture emerges when regions deleted in mastitis-prone sibs were compared with healthy sibs. 141 066 such regions were identified including 191 (0.14%) deletions observed in at least nine pairs. Those regions were annotated as exonic sequences of 46 genes (Table 2). 25 of the regions repre- sented novel deletions, while the reminder have already been reported in the Database of Genomic Variants (www.ebi.ac. uk/dgva), albeit not always as deletions. The length of the deleted regions varied between 100 and 15 200 bp and covered part of a single exon through to several exons. Seven of the affected genes potentially influence the incidence of mastitis (Figure 1). Genes with deletions found in the mastitis-prone sibs have been implicated in immune response and variations in somatic cell score, which is an

Table 2 Copy number variant regions (CNVRs) within coding sequences of the Bos taurus genome, represented by duplications present in at least nine healthy cows, but absent in their mastitis-prone half-sibs and deletions present in at least nine mastitis-prone cows, but absent in their healthy half- sibs

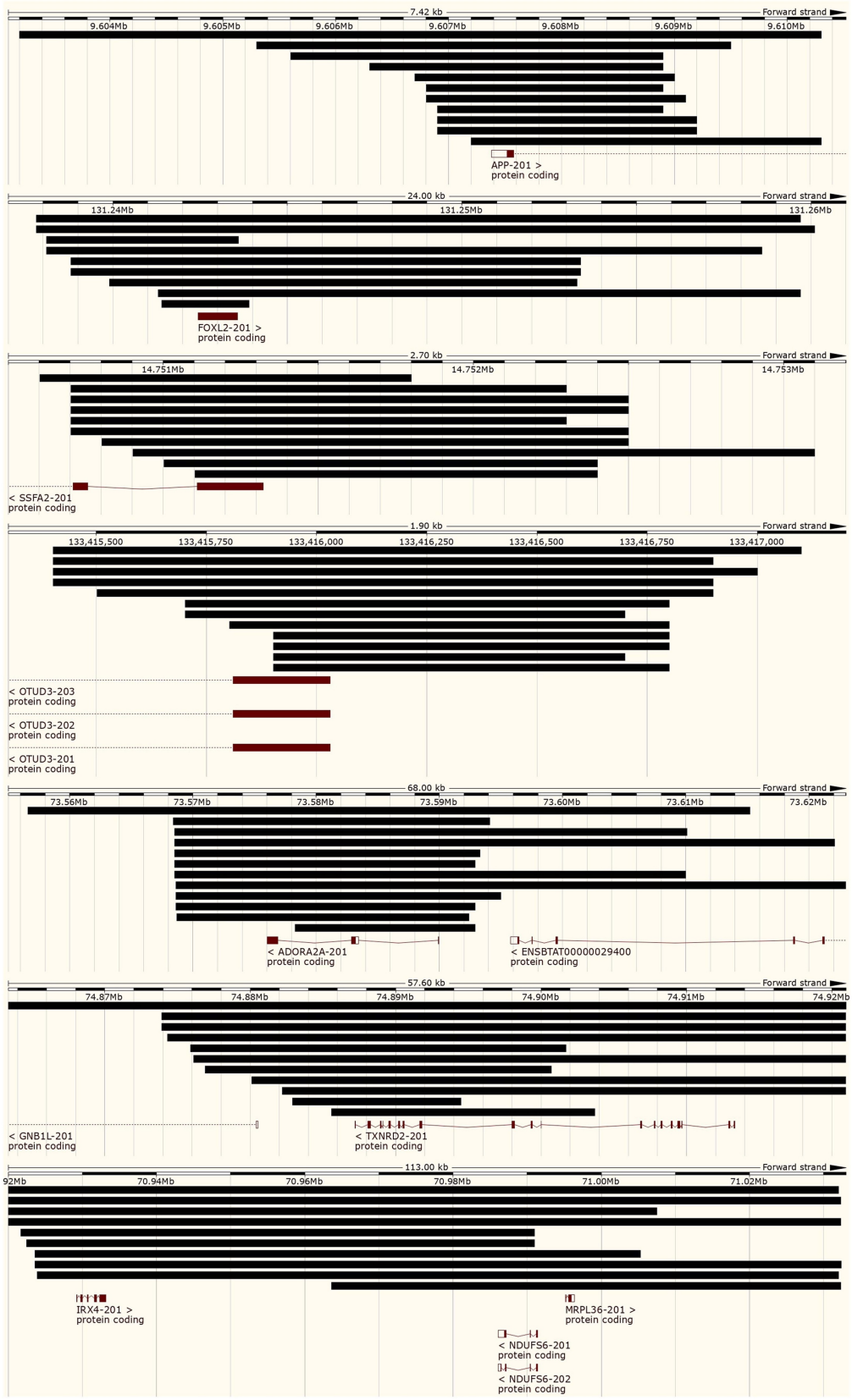
Table 2 *Copy number variant regions (CNVRs) within coding sequences of the* Bos taurus *genome, represented by duplications present in at least nine healthy cows, but absent in their mastitis-prone half-sibs and deletions present in at least nine mastitis-prone cows, but absent in their healthy half-sibs*

Chromosome	CNVR type	Begin (bp)	End (bp)	No. of differential sib pairs	Gene
1	Deletion	9607201	9 608 900	9	*APP*
1	Deletion	131241401	131 243 600	9	*FOXL2*
2	Deletion	14751101	14 751 200	9	*SSFA2*
2	Deletion	133415901	133 416 100	10	*OTUD3*
3	Deletion	16287401	16 291 900	9	*ATP8B2*
3	Deletion	34270401	34 270 900	9	*CELSR2*
4	Deletion	27855001	27 855 100	9	*TWIST1*
4	Deletion	69321501	69 324 300	9	*HOXA10*
4	Deletion	69332701	69 334 000	9	ENSBTAG00000011476
4	Deletion	69340101	69 342 800	9	*HOXA7*
4	Deletion	69353401	69 355 400	9	*HOXA5, HOXA6*
4	Deletion	77241501	77 255 800	9	*CCM2, NACAD*
5	Deletion	82468401	82 469 500	9	*KLHL42*
5	Deletion	90959701	90 961 000	9	*AEBP2*
5	Deletion	101471901	101 472 700	9	*PHC1*
6	Deletion	109355101	109 357 700	9	*UVSSA*
8	Deletion	102987401	102 987 700	9	*UGCG*
10	Deletion	32861601	32 862 000	9	*MEIS2*
10	Deletion	102885601	102 885 700	9	ENSBTAG00000045849
11	Deletion	100038001	100 038 100	9	ENSBTAG00000019513
11	Deletion	105837501	105 838 600	9	*TOR4A*
12	Deletion	32305701	32 305 701	9	*URAD*
12	Deletion	88665401	88 667 100	9	*IRS2*
13	Deletion	39202401	39 202 500	9	*SLC24A3*
13	Deletion	54345301	54 350 900	9	*SAMD10*
14	Deletion	45828001	45 828 200	9	*ZBTB10*
15	Duplication	52193201	52 196 000	9	*CHRNA10*
15	Deletion	53582301	53 582 500	9	*ARHGEF17*
16	Deletion	19500201	19 500 500	9	*KCTD3*
17	Deletion	68419101	68 423 200	9	*TFIP11, SRRD*
17	Deletion	73578301	73 586 000	9	*ADORA2A*
17	Deletion	74708501	74 708 600	10	*MRPL40*
17	Deletion	74708601	74 708 900	9	*MRPL40*
17	Deletion	74891701	74 892 600	9	*TXNRD2*
18	Deletion	54208601	54 208 700	9	*DACT3*
18	Deletion	54209401	54 209 800	9	*DACT3*
20	Deletion	70990101	70 991 000	9	*NDUFS6*
22	Deletion	1877601	1 877 800	9	*SLC4A7*
24	Deletion	5357201	5 358 300	9	*NETO1*
24	Deletion	40768401	40 768 800	9	*PTPRM*
25	Deletion	478401	479 800	9	*CAPN15*
26	Deletion	14446201	14 461 400	10	*CYP26A1, CYP26C1*
26	Deletion	25928401	25 929 100	10	ENSBTAG00000046499
26	Deletion	25929101	25 929 700	9	ENSBTAG00000046499
27	Duplication	4799101	4 799 400	9	ENSBTAG00000008519
29	Deletion	44573901	44 575 200	9	*OVOL1*

ADORA2A = Adenosine A2a receptor; *AEBP2* = AE binding protein 2; *APP* = Amyloid β precursor protein; *ARHGEF17* = ρ guanine nucleotide exchange factor 17; *ATP8B2* = ATPase phospholipid transporting 8B2; *CAPN15* = Calpain 15; *CCM2* = CCM2 scaffold protein; *CELSR2* = Cadherin, EGF LAG seven-pass G-type receptor 2; *CHRNA10* = Cholinergic receptor nicotinic α 10 subunit; *CYP26A1* = Cytochrome P450, family 26, subfamily A, polypeptide 1; *CYP26C1* = Cytochrome P450 family 26 subfamily C member 1; *DACT3* = Dishevelled-binding antagonist of β catenin 3; *FOXL2* = Forkhead box L2; *HOXA5* = Homeobox A5; *HOXA6* = Homeobox A6; *HOXA7* = Homeobox A7; *HOXA10* = Homeobox A10; *IRS2* = Insulin receptor substrate 2; *KCTD3* = Potassium channel tetramerization domain containing 3; *KLHL42* = Kelch-like family member 42; *MEIS2* = Meis homeobox 2; *MRPL40* = Mitochondrial ribosomal protein L40; *NACAD* = NAC α domain containing; *NDUFS6* = *NADH*ubiquinone oxidoreductase subunit S6; *NETO1* = Neuropilin and tolloid like 1; *OTUD3* = OTU deubiquitinase 3; *OVOL1* = Ovo-like transcriptional repressor 1; *PHC1* = Polyhomeotic homolog 1; *PTPRM* = Protein tyrosine phosphatase, receptor type M; *SAMD10* = Sterile α motif domain containing 10; *SLC24A3* = Solute carrier family 24 member 3; *SLC4A7* = Solute carrier family 4 member 7; *SRRD* = SRR1 domain containing; *SSFA2* = ITPR interacting domain containing 2; *TFIP11* = Tuftelin-interacting protein 11; *TOR4A* = Torsin family 4 member A; *TWIST1* = Twist family bHLH transcription factor 1; *TXNRD2* = Thioredoxin reductase 2; *UGCG* = UDP-glucose ceramide glucosyltransferase; *URAD* = Ureidoimidazoline (2-oxo-4-hydroxy-4-carboxy-5-) decarboxylase; *UVSSA* = UV stimulated scaffold protein A; *ZBTB10* = Zinc finger and BTB domain containing 10

Figure 1 Positions of copy number variant regions deleted in mastitis-prone siblings in Bos taurus genome covering exons of APP and FOXL2 (chromosome 1), SSFA2 and OTUD3 (chromosome 2), ADORA2A and TXNRD2 (chromosome 17), NDUFS6 (chromosome 20) genes.

APP=Amyloid β precursor protein; FOXL2 = Forkhead box L2; SSFA2 = ITPR interacting domain containing 2; OTUD3 = OTU deubiquitinase



indicator trait for mastitis. FOXL2 coding Forkhead box L2 protein (ENSBTAG00000031277), located on BTA1, had a 2 200 bp deleted region in the only exon of this gene, which was observed in nine of the mastitis-prone sibs but was not present in the healthy half-sibs. The forkhead transcription factor, encoded by FOXL2, plays a role in

inflammation processes (Moumné et al., 2008). Sperm specific antigen 2 (SSFA2; ENSBTAG00000000937), located on BTA2 had a short deletion of 100 bp in the first exon in nine mastitis- prone sibs. Variations in this gene are associated with somatic cell score, which is an indicator trait for mastitis (Strillacci et al., 2014). SSFA2 also overlaps with a QTL for bovine immunoglobulin G level (QTL ID: 20470). NADH- ubiquinone oxidoreductase subunit S6 protein gene (NDUFS6; ENSBTAE00000106646) is located on BTA20 and had a 900 bp deletion that removes a whole exon 2 and has been reported previously (Boussaha et al., 2015). This genomic region harbours a quantitative trait locus for somatic cell score (Durán Aguilar et al., 2016; Animal QTL database ID: 122069). OTU Deubiquitinase 3 protein gene (OTUD3; ENSBTAG00000017108), located on BTA2 con- tained a 200 bp deletion in 10 mastitis-prone compared with healthy sibs. This deletion removes a part of the first exon and has also been reported previously (Keel et al., 2017). OTUD3 plays a role in inflammation and is associated with inflammatory bowel disease in humans (e.g. McGovern et al., 2010). Adenosine A2a receptor gene (ADORA2A; ENSBTAG00000016944), located on BTA17 had a deletion of 7700 bp covering the whole exon 2 in nine mastitis-prone sibs. This deletion was previously reported by Hou et al. (2011). ADORA2A is involved in inflammatory diseases by modulating tissue response to inflammation (Salmon et al., 1993) and is assigned to the GO terms: inflammatory response biological process (GO:0006954) and a negative regulation of inflammatory response biological process (GO:0050728). ADORA2A is expressed at high levels in the mammary gland (Yue et alet al., 2014). Two other genes with deletions have been associated with susceptibility to bacterial infection. Thioredoxin reductase 2, mitochondrial precursor protein gene (TXNRD2; ENSBTAG00000043581) is located on BTA17 and had a 900 bp deletion in nine mastitis- prone sibs, that removes a part of the 12th exon. A CNV in this region was also reported by Hou et al. (2011). TXNRD2 is a candidate gene influencing susceptibility to S. aureus, which is one of the most common causes of mastitis, in mice (Ye et al., 2014), humans (Nelson et al., 2014) and cattle (Ghorbani et al., 2015). Amyloid β precursor protein gene (APP; ENSBTAG00000017753) located on BTA1, had a 1700 bp deletion in first exon, which was observed in nine sib-pair comparisons. The gene product is known to have bactericidal and antifungal activities and amyloid proteins have been shown to be associated with sub-CM in ruminants (Miglio et al., 2013). One CNVR, deleted in nine mastitis- prone sibs overlapped two neighbouring long non-coding RNA sequences located on BTA12 (90 641 959 to 90 643 802 bp and 90 644 418 to 90 646 031 bp) reported by Koufariotis et al. (2015).

Discussion

Our comparison of the genomes of mastitis resistant and mastitis-prone cows suggested that deletions of genomic regions were more likely to be associated with increased susceptibility to the disease. The function of genes in the affected regions was enriched in terms related to immune response and gene expression (RNA polymerase II core pro- moter proximal region sequence-specific DNA binding GO:0000978 and transcription factor activity, RNA poly- merase II core promoter proximal region sequence-specific binding GO:0000982). Similar functions were reported in the analysis of genes that were up-regulated after an artificially induced intra-mammary infection with lipopolysaccharide (Fang et al., 2017). These included, among others, genes related to RNA processing, the regulation of gene expression and the inflammatory response. There has been strikingly low reproducibility across other studies carried out to dis- entangle the genetic background of mastitis resistance in dairy cattle (e.g. Sodeland et al., 2011; Sahana et al., 2014; Olsen et al., 2016; Wang et al., 2016). The only common feature emerging were genes related to immune response. This disparity reflects the multifactorial nature of the disease, which makes identification of genes involved difficult. In our study, we reduced variation in management by working within a single cohort at the same farm and minimised the genetic variation by studying half-sib pairs, which share the same allele IBD with 0.5 probability. Moreover, the advances in technologies for whole genome sequencing have increased the proportion of variants identified in a genome, which can then be tested for association traits, by the availability of whole genome sequences with potentially (ignoring technical error in variant detection) all point and structural variants present in a genome. In this study we identified variants differing between healthy and mastitis- prone sibs and identified putative causal polymorphisms and candidate genes for CM. However, production of whole genome sequence still has a high cost and the management of the large data sets poses logistical problems. As a result studies using whole genome sequence generally have a small sample size that do not permit rigorous statistical testing of associations, so that the number of false associations (type I errors) cannot be determined. This is the case for the small data set used here, and also means that some true associa- tions could have been missed. Data quality also presents problems, as some regions could have been missed or have a sequence depth that was insufficient to determine variants. Nevertheless, the results presented here provide information on regions putatively involved in mastitis susceptibility and are a starting point for further studies.

Conclusions

The present study identified genomic regions and genes, which potentially harbour causal mutations contributing to mastitis susceptibility. Many of these regions contain struc- tural polymorphisms in the form of CNVs within genes involved in immune response, that are likely to play a role in disease susceptibility. Interestingly sequence deletions were most frequently found in the mastitis-prone sibs, suggesting that loss of function reduces resistance against CM, rather than sequence duplication, increases susceptibility to masti- tis. An interesting further extension of these findings will be estimating effects of the polymorphisms at a population- wide level.

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Supplementary material

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