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Identification of QTLs for low somatic cell count in Murrah buffaloes

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ABSTRACT

Mastitis, the most frequent and costly disease in buffalo, is the major cause of morbidity. The somatic cell count, an indirect indicator of susceptibility/resistance to mastitis, is a low heritable trait and thus a perfect candidate for marker assisted selection. Half sib families (12) were created and the somatic cell count was recorded at 3 stages of lactation during the first lactation of the 2,422 daughters belonging to 12 sires. Partial genome scan was carried out using interval mapping with different algorithms. The QTLs obtained for each half sib family were further subjected to meta analysis to identify chromosomal regions associated with somatic cell count on 8 chromosomes of buffalo. Four metaQTL regions were identified on chromosomes BBU1q, BBU8, and BBU10; 3 metaQTL regions on BBU2q, BBU9 and BBU15; 2 metaQTL regions on BBU6 and 1 on BBU7 of buffalo. Comparative genomics was used for finding out genes underlying the metaQTL regions; 1,065 genes were underlying the metaQTL regions in buffaloes assuming buffalo–cattle–human synteny. Genes (78) mapped to immune response. These genes are supposedly important candidate genes for further analysis. Gene ontology and network analysis was carried out on these genes. The genes identified belonged to immune response and defense mechanism. The QTL markers identified in the present analysis can be used in the breeding programs of buffalo to select the bulls, which are less susceptible to mastitis.

Key words: Buffalo, Interval mapping, QTLs, Mastitis, Murrah, Somatic cell count

Mastitis, the most frequent and costly disease in the dairy sector, severely affects the milk quality and renders the milk unfit for human consumption or for further processing. It also causes reduction in the milk yield in buffalo besides pain to the animal and lowers the quality of milk by changing the composition of milk. The extent of various changes in the composition of milk depends on the inflammatory response (Kitchen 1981). The somatic cells in milk are mainly the milk secreting epithelial cells which are shed from the lining of mammary gland and white blood cells that enter the mammary gland in response to infection (Sharma *et al.* 2011). The somatic cells count is indicator of both resistance and susceptibility of cows to mastitis and is an indicator to monitor the level of occurrence of subclinical mastitis (Patil *et al.* 2015). If infection occurs the major increase in somatic cell count is due to influx of neutrophils into milk to fight infection and these constitute about 90% of the count (Miller and Paape 1985, Harmon 1994). Clinical mastitis is heritable and has an unfavourable genetic correlation to milk production and thus has been included in the breeding programs in addition to milk production for avoiding increased frequency of mastitis (Heringstad *et al.* 1991, 2001). The information on clinical mastitis records is not routine under field conditions in India and these information may not be made available deliberately and thus the indirect selection using somatic cell score/ somatic cell count is recommended. The heritability of somatic cell count is low and estimated at 0.11 (Ødegard *et al.* 2003) while the genetic correlation between somatic cell count and clinical mastitis varies greatly from 0.30 to 0.97 (Emanuelson *et al.* 1988, Weller *et al.* 1992, Lund *et al.* 1999). Klugland *et al.* (2001) indicated very high genetic correlation between somatic cell count and clinical mastitis but the values were reported to be low for first lactation records. Thus the major interest in somatic cell score/somatic cell count is as an indicator to susceptibility to mastitis.

In this paper analysis was carried out to identify chromosomal regions associated with the susceptibility / resistance to mastitis using a well defined buffalo population consisting of 12 half sib families. We present partial genome scans on 8 chromosomes (of cattle, BTA) equivalent in buffaloes to identify and map the quantitative trait loci affecting the somatic cell count in buffaloes which provide us a tool for indirect selection for resistance to mastitis in buffaloes.

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MATERIALS AND METHODS

The reference family of buffaloes (animals of known pedigree and having both genotype and phenotype information recorded) was created (Vijh 2013, Vijh *et al.* 2018). The accuracy of the paternity records was authenticated using a set of DNA markers and only the daughters with confirmed paternity were recorded for phenotypes (Vijh 2014). The daughters belonging to 12 half sib families were recorded for somatic cell count during 3 stages of lactation—initial, mid and late phase. Automatic somatic cell counter was utilised for recording the somatic cell counts per ml of milk. All the records were from first lactation of buffaloes. The mean of 3 counts were utilised in this experiment. The somatic cell counts were converted into somatic cell score using log transformation ($\log_2$). The genotype data on 8 chromosomes with 79 microsatellites on these animals was generated and reported (Vijh 2013, Vijh *et al.* 2018).

A total of 12 data set were created, one each for each sire family. For a single QTL model, the standard interval mapping and Haley-Knott regression algorithm was utilised. The values of LOD score were utilised as an evidence of the existence of QTL. The statistical significance of LOD was tested using permutation test (Churchill and Doerge 1994) with 1,000 replicates. The multiple QTL models as implemented in R/qtl were used as they have increased power to detect QTL, better separation of linked QTLs and defining epistatic interactions. Full QTL model using the *fitqtl* function of R/qtl package was fitted and this also included the interaction among the qtls. The ANOVA table indicates the overall fit of the model; the LOD score obtained is relative to the null model (with no QTL). The drop one QTL model was utilised to see the effect of each identified QTL and its related interactions.

QTL meta-analysis was carried out to synthesize QTL information from 12 independent half sib family analysis results and also to refine the chromosomal region involved using Biomercator software v4.2. The QTL meta-analysis algorithm developed by Goffinet and Gerber (2000) was used. We fitted 5 models using Gaussian distribution and the best fit was determined by means of the maximum likelihood method and Akaike information content. Using the select model, consensus QTL positions were determined as the mean of QTL distribution maximizing the likelihood and confidence interval.

Once the metaQTL regions for somatic cell count with their confidence interval were known, webserver AnnotQTL (annotqtl.genouest.org) was utilised for the identification of genes underlying the QTL region (Lecerf *et al.* 2011). Each metaQTL region was taken as an input. The Buffalo-Cattle synteny based on radiation hybrid panel (Amaral *et al.* 2008) was utilised. This provided a list of genes underlying the identified QTLs for somatic cell count in buffaloes as Ensembl IDs and also provided a list of human genes assuming the synteny between cattle and human. The genes were then mapped using Reactome database available at https://reactome.org (Croft *et al.* 2014, Fabregat *et al.* 2016) to identify the genes related to immune response in cattle. Uniprot IDs associated with the identified genes were downloaded. The Uniprot ID of these genes were used as input for the webserver STRING (https://string-db.org) (Szklarczyk *et al.* 2015) to obtain network of predicted association for a particular group of proteins/genes.

RESULTS AND DISCUSSION

The somatic cell score was initially not considered to be heritable and was taken as a management problem. However, in Scandinavian countries, the records of somatic cell score and clinical mastitis revealed the heritable nature. The heritability estimates were made from designed experiments and studies obtained by Ødegard *et al.* (2003). The experiments also revealed the strong genetic correlation between somatic cell score and mastitis except for the first lactation records (Lund *et al.* 1999, Rupp and Boichard 1999, Weller *et al.* 1992). Gomez-Raya *et al.* (1998) revealed that the power of detecting QTL of a given effect is higher for low heritable traits. Further the somatic cell score is dependent on mammary gland infection and also the stage of lactation, age/breed of the individual besides parity, season and stress level. There is diurnal variation. Looking at the various factors, the present work recorded the somatic cell score at 3 different stages of lactation which covers almost all the seasons, the differences on account of parity need not be addressed since all the records were for the first lactation. A total of 2,422 animals were recorded for the trait with an average of 201.83 daughters per sire. The sire family, and number of daughters in the sire family with somatic cell score are presented in Table 1.

The scans of 8 chromosomes studied in the experiments revealed large number of QTLs for somatic cell count in buffaloes (Table 2). Total 5 interactions among the QTLs located on different buffalo chromosomes were statistically significant. Out of these five interactions, three had chromosome number 1 and 3 involved chromosome BTA4 and 2 involved BTA 14 chromosome. The representative

Table 1. Sire wise distribution of daughters for mean of somatic cell score

Sire	No. of daughters	Mean±SE
Sire 1	334	3.05±0.09
Sire 2	186	3.33±0.12
Sire 3	93	2.96±0.17
Sire 4	243	3.27±0.09
Sire 5	288	3.17±0.10
Sire 6	80	3.09±0.21
Sire 7	360	3.09±0.07
Sire 8	293	3.24±0.09
Sire 9	232	2.73±0.09
Sire 10	166	3.08±0.14
Sire 11	93	2.82±0.14
Sire 12	54	2.81±0.19

Table 2. Significant QTL locations for somatic cell count for 12 half sib families using Interval Mapping (Haley-Knott regression and extended Haley-Knott regression) as implemented in R/qtl

	Chromosome	Position	LOD	% var	F value	P value (F)	Significance
Sire 1	9	49.2	1.19182	1.26379	2.39414	0.0931	P
	1@32.1:4@93.0		1.99465	2.12688	2.0146	0.0925	P
Sire 2	1	14.1	2.44141	4.6275	5.203	0.006427	**
	6	36	1.45371	2.7216	3.0601	0.049521	*
	9	46.2	5.8735	11.6243	4.3567	0.00041	***
	14	65.1	5.82566	11.5226	4.3186	0.000446	***
	9@46.2:14@65.1		4.63199	9.0244	5.0734	0.000697	***
Sire 3	1	56.1	4.5661	4.0951	5.3278	0.00867	**
	2	16.8	2.8777	2.472	3.2162	0.05016	P
	3	84.3	3.4519	3.0088	3.9146	0.02761	*
	4	84	7.1645	6.8738	2.981	0.01621	*
	7	24	3.2055	2.7766	3.6124	0.03568	*
	7	123	2.7379	2.3436	3.0491	0.05802	P
	9	46.2	2.5005	2.1276	2.7681	0.07425	P
	14	86.1	3.2606	2.8283	3.6797	0.03369	*
Sire 4	1	152.1	2.9159	4.4826	2.026	0.0634	P
	4	30	1.4579	2.2103	2.998	0.052	P
	7	123	1.342	2.0323	2.756	0.0658	P
Sire 5	3	63.3	1.1491	1.6523	2.4571	0.0876	P
	9	37.2	1.5074	2.1738	3.2325	0.041	*
Sire 6	4	117	4.4285	16.766	2.952	0.01352	*
	7	126	2.8852	10.432	5.511	0.00631	**
	14	77.1	3.9269	14.645	2.579	0.02719	*
	4@117.0:14@77.1		3.3003	12.081	3.191	0.01911	*
Sire 7	1	65.1	2.5253	2.9732	1.855	0.0879	P
Sire 8	1	17.1	7.573673	8.641615	5.225046	4.37E–05	***
	1	59.1	1.950581	2.128245	3.860452	0.0223	*
	4	102	7.931921	9.076413	5.487942	2.36E–05	***
	1@17.1:4@102.0		6.814774	7.728601	7.009506	2.32E–05	***
Sire 9	9	55.2	1.475	2.5737	3.194	0.043	*
Sire 10	2	10.8	1.7328	3.3537	3.4226	0.0354	*
	2	19.8	3.5277	7.002	2.3819	0.032	*
	9	64.2	4.2642	8.5521	2.9092	0.0105	*
Sire 12	1	47.1	7.069	16.771	4.55	0.00182	**
	2	91.8	2.498	4.812	3.917	0.02976	*
	3	33.3	6.992	16.528	4.484	0.002	**
	4	21	1.662	3.087	2.513	0.09644	P
	7	126	3.282	6.547	5.329	0.00987	**
	9	13.2	3.006	5.923	4.821	0.01456	*
	14	44.1	3.777	7.703	6.27	0.00492	**
	1@47.1:3@33.3		4.317	9.022	3.672	0.01399	*

***0.001; **0.01; *0.05; P, Probable QTL.

chromosomal scans of chromosome 1 and chromosome 9 are depicted (Supplementary Fig. 1). Infact chromosome 6 harbours genes for not only proteins and fats but also for milk yield and somatic cell score in buffaloes. Total 36 QTLs were found associated with somatic cell count in the 12 halfsib families analysed. The QTLs identified in each of the halfsibs families were independent of one another. QTLs' identification is dependent on the heterozygosity of the sire on particular marker loci and thus the different QTLs may be identified in different sire families.

The analysis revealed 36 QTLs for somatic cell score in buffaloes. There have been no reports of QTL identification for any of the economic traits in buffaloes. As somatic cell score is associated with clinical mastitis and the results are available in cattle identified QTL markers are of large economic significance. Control of mastitis is of great importance since it shall make the dairy production more cost effective by reducing the morbidity in buffaloes. It shall also help in reduction of the use of antibiotics and subsequent indirect consumption by humans. It will also improve the animal welfare. These markers are now a part of breeding programs in all the developed countries of the world. In this study, we identified QTL markers on 8 chromosomes for somatic cell count utilising 12 halfsib families. We identified the QTLs for somatic cell count on all the 8 chromosomes although in different sire families.

Table 3. Locations of metaQTL regions (chromosome wise) for somatic cell count along with their confidence interval

Chromosome (BTA)	BBU Chromosomes	AIC value	Mean position 1 (C.I.)	Mean position 2 (C.I.)	Mean position 3 (C.I.)	Mean position 4 (C.I.)
1	BBU1q	52.04	15.42 (12.85–18.0)	47.07 (42.93–51.22)	75.81 (72.5–79.11)	154.67 (146.11–155.0)
2	BBU2q	24.03	10.77 (9.0–16.77)	19.77 (16.17–23.37)	90.77 (85.84–99.77)	–
3	BBU6	17.15	35.76 (28.34–40.34)	82.95 (79.9–86.0)	–	–
4	BBU8	35.7	12.54 (7.0–21.0)	25.99 (18.0–32.0)	99.69 (95.53–103.85)	111.0 (104.0–117.16)
6	BBU7	5.71	35.39 (32.0–42.0)	–	–	–
7	BBU9	25.65	24.39 (21.0–28.0)	124.0 (114.0–126.24)	126.11 (124.62–127.59)	–
9	BBU10	51.23	13.2 (10.2–20.2)	22.2 (16.2–25.2)	46.76 (45.1–48.43)	66.2 (59.51–70.2)
14	BBU15	25.98	43.12 (34.12–48.12)	66.12 (62.12–75.12)	92.43 (88.41–96.46)	–

Values in parenthesis represent the confidence interval of the metaQTL. BTA implies *Bos taurus* chromosomes and BBU implies *Bubalus bubalis* chromosomes.

The positions of the QTL regions may differ from family to family and need to be further analysed to find consensus regions. The meta analysis of the identified QTL regions for somatic cell count in buffaloes was carried out on QTLs on 8 chromosomes of buffaloes. The selection of the model from the 5 models tested for the analysis was based on Akaike Information Content. The meta analysis of the QTLs across 12 families of buffaloes are depicted in Table 3.

Total 24 metaQTL regions were identified (Table 3). The chromosome BBU1 q, BBU8 and BBU10 had four metaQTL regions each while BBU2 q, BBU9 and BBU15 had three metaQTL regions on each chromosomes. Similarly, BBU6 revealed two and BBU7 revealed one metaQTL region (Fig. 1). Since no information is available on the QTLs for somatic cell count in buffaloes we compared the chromosomal positions of buffalo QTLs with those of cattle QTLs for the trait assuming a high degree of synteny between cattle and buffalo chromosomes as reported by Amaral *et al.* (2008). The QTL region at mean position 15.42 with a span of 12.85 to 18.00 cM on chromosome was similarly reported for the trait by Strillacci *et al.* (2014). The second metaQTL position on chromosome arm of BBU1 q was on 47.07 with confidence interval of 42.93 to 51.22 and has also been reported by Cole *et al.* (2011). The third mean position on the same chromosome was at 75.81 and had a confidence interval of 72.5 to 79.11. QTLs have been reported by several authors in cattle between the same confidence interval (Bennewitz *et al.* 2003, Baes *et al.* 2010 and Chen *et al.* 2011).

Similarly, the meta-analysis revealed three meta QTL positions on BTA 2 (equivalent BBU2 q). The metaQTL region identified in buffalo at mean position 10.77 with a confidence interval of 9.0 to 16.77 has been reported in cattle. The second mean position of 19.77 with confidence interval of 16.17 to 23.37 has been reported by several workers in cattle (Rupp and Boichard 2003, Cole *et al.* 2011 and Strillacci *et al.* 2014). Similarly for chromosome BTA 3 (BBU6), two meta QTL regions were identified. The first mean position is also reported in literature by Liu *et al.* (2012) while the second mean position is reported by Rupp and Boichard (2003), Cole *et al.* (2011) and Strillacci *et al.* (2014). We identified 4 metaQTL regions on chromosome BBU8 (BTA4) and out of these four locations, the metaQTL position at 12.54 has been reported by Cole *et al.* (2011) and Wijga *et al.* (2012) while the location at 25.99 with a confidence interval between 18.0 to 32.0 has been reported by several authors in taurine cattle (Rupp and Boichard 2003, Daetwyler *et al.* 2008 and Cole *et al.* 2011). The mean position at 99.69 with a confidence interval of 95.83 to 103.85 has been reported in cattle (Tal- Stein *et al.* 2010, Cole *et al.* 2011 and Wang *et al.* 2015). The fourth metaQTL region has been reported in cattle by Strillacci *et al.* (2014). For the chromosome BTA 6 (BBU7), we identified only one metaQTL region at 35.39 mean position which has also been identified to harbour genes for somatic cell count by several authors (Daetwyler *et al.* 2008, Alain *et al.* 2009, and Tal-Stein *et al.* 2010). Incidentally the region also has QTLs for high milk yield in cattle as reported by Liu *et al.* (2004), Khatib *et al.* (2007), Doran *et al.* (2014). Same region has been reported in association studies of milk and somatic cell score by Meredith *et al.* (2012). There is unfavourable genetic correlation between high milk production and increased frequency of mastitis. We assume such high correlation between high somatic cell count and milk yield might exist in buffaloes too. It was thus an expected result that some of the QTLs for high somatic cell count shall be positioned close to milk production traits. The present analysis identified 3 metaQTL regions on BTA 7 (BBU9) which have also been identified in cattle by various authors. The first metaQTL with mean position at

24.39 has been reported by Rupp and Boichard (2003), Ron *et al.* (2004), Cole *et al.* (2011), Strillacci *et al.* (2014) and El-Halawany *et al.* (2017). Similarly, the mean position at 124.0 has been reported by Tal-Stein *et al.* (2010) and mean position at 126.11 by Heyen *et al.* (1999). The chromosome BTA9 (BBU10) revealed four metaQTL regions. The first mean position at 13.2 not reported in literature while the mean position at 22.2 has been reported by Sahana *et al.* (2008). Position at 46.76 has been reported by Boichard *et al.* (2003) and Cole *et al.* (2011). The fourth mean position on BBU10 at 66.2 has been reported by Tal-Stein *et al.* (2010) in cattle. The eight chromosome (BBU15) equivalent to BTA 14 had three metaQTL locations identified and each has been reported in literature for the trait of interest. The first location at mean position at 43.12 with a confidence interval between 34.12 and 48.12 has been reported by Zhang *et al.* (1998), Rupp and Boichard (2003), and Cole *et al.* (2011) for cattle. Mean position of the metaQTL at 66.12 has been reported in cattle by Lund *et al.* (2007), Daetwyler *et al.* (2008) and Cole *et al.* (2011). The mean position at 92.43 also have comparative region identified in cattle by Rodriguez-Zas *et al.* (2002), Bennewitz *et al.* (2003) and Wang *et al.* (2015). Thus most of the identified metaQTL regions have been found to have similar locations as reported for QTL regions in cattle for which extensive work has been carried out over the past two decades especially for Holstein and Jersey breeds of cattle.

It is however known that the detection of QTLs is an inexact science and thus require the verification in different populations. Taking into consideration the above statements, we not only identified the QTLs in 12 half sib families but also carried out the metaQTL analysis to find the consensus regions affecting the somatic cell count in buffaloes. Several strategies have been put forward for the confirmation of suggestive QTLs for the traits of interest (Spelman and Bovenhuis 1998, Georges 1999). One of the most common ones are to check for the genes underlying QTL regions in the species. In our case, a well documented and curated buffalo sequence is not available while there is large degree of synteny between cattle and buffalo chromosomes (Amaral *et al.* 2008). Thus we utilised the tools of comparative genomics to find out the genes underlying QTL regions in buffaloes. The total genes identified under the metaQTL regions for somatic cell count were 1065 (Fig. 2). The identified genes which mapped the immune related genes in the Reactome database (https://reactome.org/) were 78. The list of the genes identified and related to the immune response or immune system are given in Table 4.

The genes that underlie QTLs for somatic cell count in buffaloes and related to immune response and defense mechanism included 6 Interleukin genes, viz. IL12RB2, IL23R, IL6, IL13, IL14 and IL7. Besides there were cluster differentiation factors, viz. CD28, CD58 and CD53 (Chan *et al.* 1988, Zola *et al.* 2007). In addition there were genes related to nuclear pore complexes. The protein encoded by IL12RB1 is a type I trans-membrane protein identified as a subunit of the interleukin 12 receptor complex. The expression of this gene is up-regulated by interferon gamma in Th1 cells, and plays a role in Th1 cell differentiation. The up-regulation of this gene is associated with a number of infectious diseases (Kato-Kogoe *et al.* 2016). While Interleukin 6 (IL-6), promptly and transiently produced in response to infections and tissue injuries, contributes to host defense through the stimulation of acute phase responses, hematopoiesis, and immune reactions. Although its expression is strictly controlled by transcriptional and posttranscriptional mechanisms, non-regulated continual synthesis of IL-6 plays a pathological effect on chronic inflammation and autoimmunity (Woo and Humphries 2013, Tanaka *et al.* 2014). Interleukin 13 (IL-13) has biological activities on B cells, monocytes/macrophages and endothelial cells. IL-13 is primarily produced by T_H2 cells, but it is also secreted by other T helper cell subsets $CD8^+$ T cells, mast cells, eosinophils and basophils following activation. IL-13 induces proliferation and immunoglobulin E (IgE) synthesis by B cells. IL-13 inhibits production of proinflammatory cytokines and chemokines by monocytes/macrophages indicating that IL-13 also has important anti-inflammatory properties. IL-4 is also called the 'prototypic immunoregulatory cytokine.' Like many cytokines, it can affect a variety of target cells in multiple ways. IL-4 has an important role in regulating antibody production, hematopoiesis and inflammation, and the development of effector T-cell responses (Mitchell *et al.* 2017). IL7 encodes a protein-a cytokine important for B and T cell development (Elkassar and Gress 2010). CD58, or lymphocyte function-associated antigen 3 (LFA-3), is a cell adhesion molecule expressed on antigen presenting cells (APC), particularly macrophages (Jordan *et al.* 2014). It binds to CD2 (LFA-2) on T cells and is important in strengthening the adhesion between the T cells and Professional APC. Leukocyte surface antigen CD53 is a protein encoded by this transmembrane 4 superfamily. This encoded protein is a cell surface glycoprotein that is known to complex with integrins. It contributes to the transduction of CD2-generated signals in T cells and natural killer cells and has been suggested to play a role in growth regulation. CD28 (Cluster of Differentiation 28) is one of the proteins expressed on T cells that provide co-stimulatory signals required for T cell activation and survival. T cell stimulation through CD28 in addition to the T-cell receptor (TCR) can provide a potent signal for the production of various interleukins (IL-6 in particular). The gene is closely associated with Toll like receptor genes. CD28 is the only B7 receptor constitutively expressed on naive T cells. The *ITGB2* gene provides instructions for making one part (the β2 subunit) of at least four different proteins known as β2 integrins. Signaling through the β2 integrins triggers the transport of the attached leukocyte across the blood vessel wall to the site of infection or injury. CTLA4 or CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), also known as CD152 (cluster of differentiation 152), is a protein receptor that, functioning as an immune checkpoint, downregulates immune

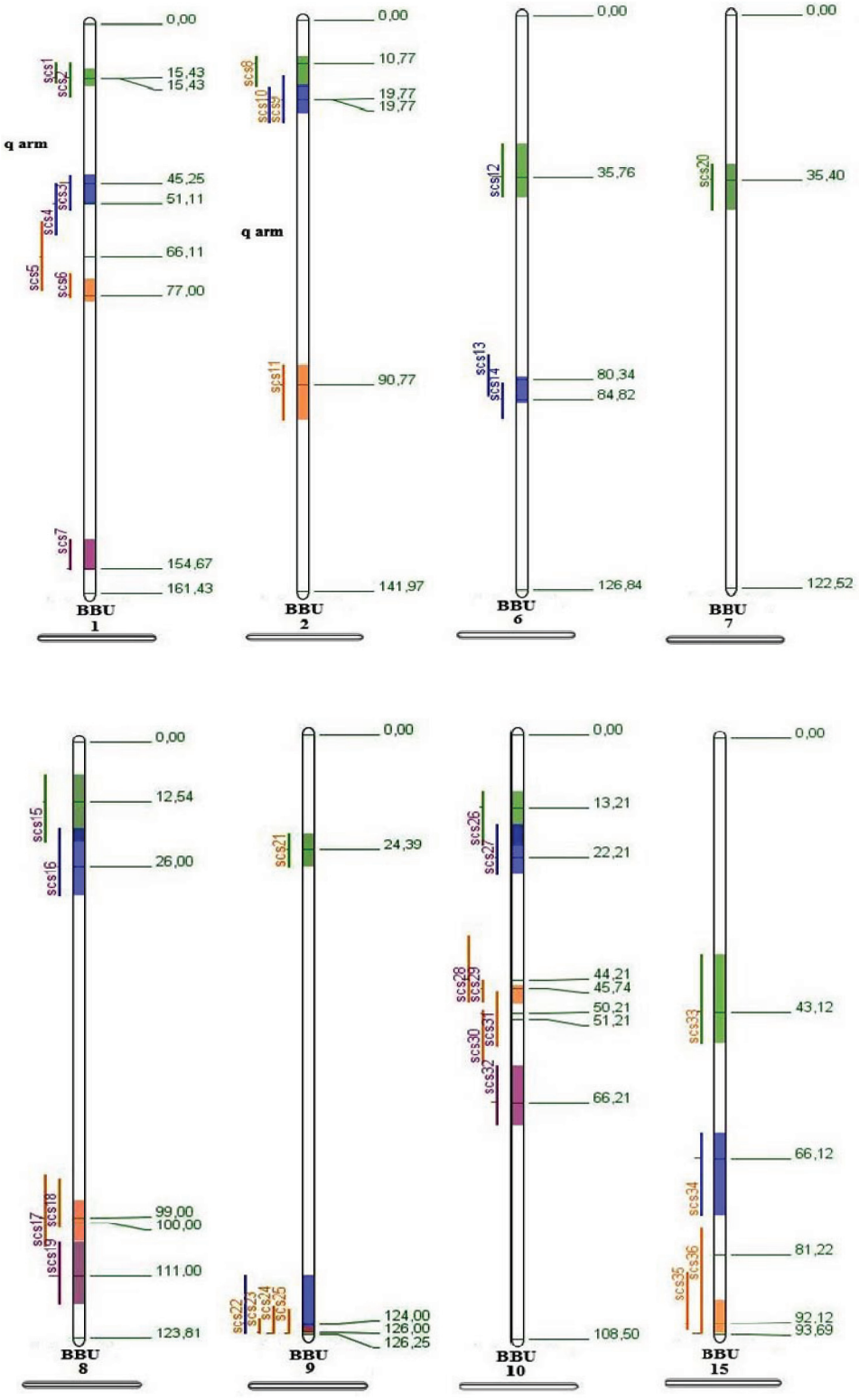

Fig. 1. The metaQTL positions for somatic cell count on 8 chromosomes for buffalo.

responses. CTLA4 is constitutively expressed in regulatory T cells but only upregulated in conventional T cells. It acts as an "off" switch when bound to CD80 or CD86 on the surface of antigen-presenting cells. NUP 35 functions as a component of the nuclear pore complex (NPC). NPC components, collectively referred to as nucleoporins (NUPs). These NUPs can play the role of both NPC structural components and of docking or interaction partners for transient association of nuclear transport factors. May also play a role in the association of MAD1 with the NPC (Doye and Hurt 1997). PTPN22 acts as negative regulator of T-cell receptor (TCR) signaling and positively regulates

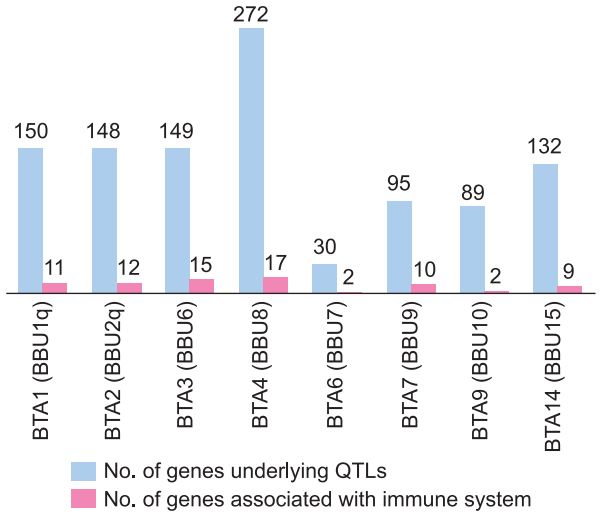

Fig. 2. Total number of genes underlying metaQTL regions and genes associated with somatic cell count.

toll-like receptor (TLR)-induced type 1 interferon production. CSF2 is a gene which encodes a cytokine that stimulates the growth and differentiation of hematopoietic precursor cells from various lineages, including granulocytes, macrophages, eosinophils and erythrocytes.

The interaction among the genes is presented in Supplementary Fig. 2. To find out the overall ontology of these genes we downloaded the GO IDs of the genes and carried out the analysis using REVIGO webserver (http:// revigo.irb.hr/) to analyse the GO terms based on their dispensability (Supek *et al.* 2011). The results of the GO analysis revealed cell activation, glucose transport, immune system processes, wound healing, regulation of cell killing and the like biological functions. In the cellular component analysis, the membrane, cytosol extracellular region, plasma membrane etc. were the ontology terms in cellular component.

Thus it is observed that a large number of genes which are associated with the immune response in humans and probably also in cattle and buffalo which are strong candidate genes to work on. These genes might be directly or indirectly associated with the somatic cell count and their

Table 4. Genes associated with somatic cell count identified using Reactome database

Chromosomes	Submitted entities found	Mapped entities	HGNC names
BTA1 (BBU1q)	ENSBTAG00000017060	P05107	ITGB2
	ENSBTAG00000004777	P60604	S100B
	ENSBTAG00000018186	Q13191	PDXK
	ENSBTAG00000004833	P17858	NIT2
	ENSBTAG00000021901	P04271	TRPM2
	ENSBTAG00000010658	O75144	PFKL
	ENSBTAG00000020787	P22792	PIK3R4
	ENSBTAG00000014880	Q99570	N/A
	ENSBTAG00000032656	O94759	CPN2
	ENSBTAG00000013057	Q9NQR4	CBLB
	ENSBTAG00000048090	O00764	UBE2G2
BTA2 (BBU2q)	ENSBTAG00000019929	P16410	N/A
	ENSBTAG00000020527	P06756	IDH1
	ENSBTAG00000013170	Q9Y2A7	CTLA4
	ENSBTAG00000009859	P16220	N/A
	ENSBTAG00000002295	O75874	ATF2
	ENSBTAG00000007445	P10747	CD28
	ENSBTAG00000009777	P63165	NUP35
	ENSBTAG00000015718	Q14790	CASP8
	ENSBTAG00000001585	P13612	WIPF1
	ENSBTAG00000033662	P15336	NCKAP1
	ENSBTAG00000005474	Q8NFH5	N/A
	ENSBTAG00000009256	O43516	ITGA4
BTA3 (BBU6)	ENSBTAG00000014710	P09603	RAP1A
	ENSBTAG00000019617	Q9Y2R2	PTPN22
	ENSBTAG00000000283	Q5VWK5	CSF1
	ENSBTAG00000006466	P28066	CD53
	ENSBTAG00000040131	P19397	CD58
	ENSBTAG00000003147	O43865	N/A
	ENSBTAG00000046797	P19256	NRAS
	ENSBTAG00000020641	Q99665	PSMA5
	ENSBTAG00000009455	P36871	IL12RB2
	ENSBTAG00000019011	P52907	N/A
	ENSBTAG00000014295	Q93033	CAPZA1
	ENSBTAG00000014983	Q9UKW4	IL23R
	ENSBTAG00000018893	P62834	AHCYL1
	ENSBTAG00000031575	P23458	VAV3
	ENSBTAG00000018203	P01111	N/A
BTA4 (BBU8)	ENSBTAG00000014921	O43451	IL6
	ENSBTAG00000007572	Q8NHE4	N/A
	ENSBTAG00000011127	Q9Y574	NUP205
	ENSBTAG00000027134	Q8WXI3	DYNC1I1
	ENSBTAG00000006022	Q16864	N/A
	ENSBTAG00000020453	Q13616	CLEC5A
	ENSBTAG00000007442	P35030	AKAP9
	ENSBTAG00000021565	P19801	N/A
	ENSBTAG00000004263	Q9HBG4	ATP6V0A4
	ENSBTAG00000021761	P15056	BRAF
	ENSBTAG00000005419	Q99996	AOC1
	ENSBTAG00000008736	Q9NY25	CUL1
	ENSBTAG00000018185	Q92621	ASB4
	ENSBTAG00000046152	P05231	MGAM
	ENSBTAG00000016752	O14576	ASB10
	ENSBTAG00000018159	O15504	CASP2
	ENSBTAG00000016014	P42575	NUPL2
BTA6 (BBU7)	ENSBTAG00000020538	Q8IVU3	N/A
	ENSBTAG00000020536	Q9UII4	HERC6
BTA7 (BBU9)	ENSBTAG00000015953	P10914	IL13
	ENSBTAG00000015957	P36507	IL4
	ENSBTAG00000020446	Q14213	THOP1
	ENSBTAG00000031235	Q9UM11	N/A
	ENSBTAG00000001570	Q9Y496	CSF2
	ENSBTAG00000024450	P35225	N/A
	ENSBTAG00000031231	P04141	IRF1
	ENSBTAG00000012829	P52888	EBI3
	ENSBTAG00000025477	P05112	N/A
	ENSBTAG00000031387	P05113	FZR1
BTA9 (BBU10)	ENSBTAG00000002625	Q9NV96	MAP3K7
	ENSBTAG00000005100	O43318	TMEM30A
BTA14 (BBU15)	ENSBTAG00000015536	Q9Y6Y9	CPNE3

QTL regions have been designated in the present study. Meta-QTL regions associated with somatic cell count were identified based on the analysis of 12 half sib families. The genes underlying the QTL regions identified the genes associated with the immune response which can be probable candidate genes on which further work can be pursued. The somatic cell score that has a low heritability (Rupp and Boichard 2003) and is one of important traits for which the markers can be of great success for selection of bulls for low somatic count. The polygenic nature of mastitis make it challenging to identify the putative causal genes associated with variation in mastitis resistance. Once the markers have been identified, this shall help in selection and propagation of buffaloes which are less susceptible to mastitis.

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