GENOME-WIDE ASSOCIATION FOR UDDER COMPOSITION TRAITS IN *BOS INDICUS – BOS TAURUS* COWS

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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May 2016

Major Subject: Animal Breeding

ABSTRACT

The objective of this study was to conduct a genome-wide association study for udder traits for crossbred cows. Average teat diameter, average teat length, and udder support score were evaluated in cows (n = 295) from 13 full-sib embryo transfer Nellore-Angus F_2 families and 4 half-sib natural service families sired by the same bulls in central Texas. Cows were analyzed together as paternal half sibs. Effects included in the analyses were cow age nested within birth year-season combinations. Cow was included as a random effect. Repeated measures analyses were conducted to generate residuals for each cow then averaged per cow for each trait and used as individual phenotype. Association analyses for teat diameter and length on each quarter, the average of the four quarters, and udder support score were conducted.

A total of 15 associated ($-\log10[\text{FDR}] \geq 1.3$) markers were detected. Udder support had 15 significant SNP detected, exclusively within a 26.4 Mb region on BTA 5 (21.6 to 48.1 Mb), while average teat diameter had one associated marker, also located on BTA 5. No SNP associations were detected for average teat length or any individual teat traits. These results identified associated markers within genes that have an involvement in the development and regulation of the mammary system and suggest that BTA 5 is significant for udder traits in beef cattle.

DEDICATION

To the loving memory of my father, John Robert Williams ‘75.

He instilled a passion for learning and Texas A&M deep within my soul. His love, encouragement, belief in and nurturance of my abilities started me on this journey. This exists because of him.

ACKNOWLEDGEMENTS

I would like to thank my advisory committee: Dr. Jim Sanders, Dr. Andy Herring, Dr. David Riley, and Dr. Chris Skaggs. Thank you all for your assistance and mentorship throughout my graduate education. I also thank you for entrusting me with numerous teaching opportunities. Teaching students in subjects that formed the foundation of my Animal Science education will forever be my favorite aspect of my time as a graduate student.

Thank you especially to my committee chair, Dr. Sanders. The interest you sparked in me for Animal Breeding when I was just an undergrad sitting in the second row of your class lead me to this field of research and the path I am on today. You pushed me to achieve. You knew I could always be better, and your belief in me kept moving me forward.

Dr. Riley, your door has always been open to me. After countless conversations filled with advice, insight, and encouragement for both my research and life outside of school, I have finally reached the end. I thank you for your ability to take a cluster of sentences and statistics and see meaning. Without your guidance, generosity, and never ending patience I would have been lost. I will be forever grateful.

I must also thank the other graduate students in our department office, 434. No one quite understands life on the fourth floor like you. You gave me friendship and advice when I needed it most. To those who went before me – thank you for your

direction and for teaching me the ropes. To those who follow – keep working, your time will come, and some day you might get one of the desks in the back.

Most importantly, I would like to thank my friends and family who have always believed in me and given me the love and inspiration needed to achieve my goals. I could not ask for a better support system.

Thank you to my husband, Ross. Your patience astounds me. You have been my eternal champion. Even though you were only a part of this team for the latter half of this journey, it was by far the most emotional and demanding part, and you helped me cross the finish line. You were always ready with encouragement and shared your own strength and courage when I had lost mine. My success was our success. You inspire me to be better every single day, and I'm grateful to have had you by my side in this adventure.

Mom, you've been here for it all: the highs and the lows, the smiles and the tears, the beginning and the end. You have given me unconditional love and support, and constant reassurance that I was on the right path and that I should always be proud of myself. You taught me about hard work and dedication and made me believe I can be successful in anything I choose. Always ready with an ear to listen when I needed it most, you shared in the many uncertainties, challenges, and sacrifices in completing this research. This would not have been possible without your love. I cannot thank you enough.

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INTRODUCTION

The complexities of udder conformation traits have been a source of research potential and interest for many years. Calf survival, growth, and cow longevity are dependent on highly variable udder conformation traits. Minimal results from genetic evaluation of udder characteristics have been reported from within lines or among families, though there is an apparent tendency for *Bos indicus* influenced, namely Brahman, cows to exhibit problematic udder and teat characteristics (Cartwright, 1980) which can have adverse effects on a calf's ability to nurse (Wythe, 1970). There is evidence of genetic differences for udder conformation (Rohrer et al., 1988; Riley et al., 2001; Hearnshaw et al., 2002; MacNeil and Mott, 2006) with heritability reported as moderate (Fuerst-Waltl et al., 1998; Chrystal et al., 1999; Schrooten et al., 2000; MacNeil and Mott, 2006; Bradford et al., 2015). According to Frisch (1982), the size, overall condition, and health of a cow's udder as she ages affects the survival and growth of her calves. The economic impact on the producer justifies selection against any udder or teat defect that negatively affects calf growth rate due to reduced milk availability to the calf.

Differences in conformation have intrigued breeders since the beginning of history as it relates to livestock (Freeman, 1976). Different traits have varying value among livestock producers, and selection criteria are based heavily on specific breeds as well as operation and production goals. For instance, dairymen most frequently cull cows for reasons associated with udder conformation (Freeman, 1976) and reproduction

(Bascom and Young, 1998). Most breeds of beef cows are expected to reach peak calf production between the ages of 5 to 10 years. However, poor udder and teat conformation may reduce the ability of the cow to remain in the herd over time, even if that cow produces an adequate quantity of milk. The productive life of a cow is limited by physical soundness as she ages (Riley et al., 2001) and culling of beef cows is motivated by poor udder conformation (Rohrer et al., 1988; Arthur et al., 1992). Given that cows seldom die of old age, a cow is maintained in the herd as long as she is productive. Greater longevity translates into less culling, and this, in turn, reduces the associated costs of herd replacement and increases the proportion of the high-producing mature cows in the breeding herd (Arthur et al., 1992). According to the National Animal Health Monitoring System Beef 2007-2008 survey (NAHMS Beef 2007-2008, 2010), in general, the percentage of operations that sold at least one cull cow in 2007 for physical unsoundness, bad eyes, udder problems, or producing a poor calf increased as herd size increased. For operations across all herd sizes, over one-half (52.6 percent) of cows that were culled were 10 yr of age or older, followed by 31.8 percent for cows aged 5 to 9 yr.

Selection in animals and plants for quantitative traits of economic importance is traditionally based on phenotypic records of the individual and its ancestors, siblings, offspring, or a combination of these relatives. According to Garrick (2011), conventional livestock breeding programs may delay selection decisions well beyond puberty so that phenotypic performance can be observed on progeny or other relatives. Meuwissen (2001) justified molecular genetics research on livestock and crop species with the expectation that information at the DNA level will lead to faster genetic gain than that based on phenotypic data alone. Genome-wide association studies (GWAS) examine the entire genome in search of associations of DNA variants such as single nucleotide polymorphisms (SNP) with phenotypic variation. These associations determine the location of quantitative trait loci (QTL), loci involved in the control of quantitative traits (Chamberlain, 2012). Molecular-based information has long held promise to improve the prediction of breeding values of young animals by first using phenotypic markers, second using microsatellite markers, and most recently using increasing densities of SNPs (Garrick, 2011).

Utilizing data available from markers in selection can increase the accuracy and efficiency of a breeding program when weighed against traditional breeding schemes (Gray et al., 2012). Markers with large effects on traits of economic importance may be used to identify regions of the genome that merit further study to identify causative mutations (Cole et al., 2009). No association analyses for udder traits in beef cows have been reported to date, therefore, the objective of this project was to conduct a genome-wide association study for udder traits of *Bos indicus – Bos taurus* cows.

LITERATURE REVIEW

Beef Cattle Longevity and the Influence of Udder Traits

The majority of commercial beef cattle herds in the southern United States are composed of crossbred cows with some degree of Brahman lineage blended with Continental, British and possibly dairy breeding (Franke, 1980). Zebu cattle are generally considered to belong to the *Bos indicus* (sometimes referred to as *Bos taurus indicus*) sub-species and European cattle to the *Bos taurus* (sometimes referred to as *Bos taurus taurus*) sub-species (Sanders, 1980). The prevalent beef breeds in the United States have traditionally been *Bos taurus* cattle due to heavy European influences. There are limitations on performance of *Bos taurus* cattle in sub-tropical regions due to lack of tropical adaptation. Crossbreeding between *Bos taurus* and *Bos indicus* cattle has been widely practiced in subtropical regions due to the benefits of heterosis and breed complementarity for reproduction, growth, and carcass traits (Kim et al., 2003). Sanders (1980) wrote about the history and characteristics of several *Bos indicus* breeds of cattle, including Nellore. The Nellore breed has its origins in India and, along with Guzerat and Gir, has probably had the most influence on Zebu cattle breeding in the United States.

Rohrer et al. (1988) reported data from 498 cows of 15 breed-types at Texas A&M University Research Center at McGregor. These cows were produced in a five-breed diallel including Angus, Brahman, Hereford, Holstein, and Jersey. Differences in longevity, average life span, and reasons for herd removal were analyzed over approximately 14 years. Longevity of crossbred cows was greater than that of purebred cows, and generally, Brahman crossbreds had the largest estimates for longevity. However, teat shape was a problem in cows with Brahman breeding. Bailey (1991) found that F_1 *Bos indicus* females were outstanding in longevity, thus supporting the findings by Rohrer et al. (1988).

Increased longevity reduces costs of replacing females (Cundiff et al., 1985). Kersey DeNise et al. (1987) reported scores for udder capacity and udder shape on 3- to 10-yr-old Hereford cows. Pendulous udders that were low to the ground were prone to mastitis. High, wide, and firmly attached udders with appropriate teat size and placement were found to be favorably correlated to longevity. Results from Kuhn et al. (2003) suggest that loci with influence on udder health may also contribute to the genetic variance of longevity.

Udder Support

Variations in udder characteristics have a significant impact on both the beef and dairy production industries, but with different selection emphasis. The udder characteristics of dairy cows can be used in beef cattle studies as a means of comparison, nevertheless.

A pendulous udder combined with large teat diameter or teat length challenges the nursing ability of most calves (Riley et al., 2001). Arthur et al. (1992) found that culling based on udder problems was of greatest significance in cows aged 6 yr and older.

Edwards (1982) wrote that the most important factor in determining the time to first suckling was the udder conformation of the dam. Ensuring calves stand and are able to receive colostrum is vital to the health and well being of the calf. After birth, the ability of the calf to absorb colostral Ig decreases progressively with time (Kruse, 1970b), and immunoglobulin concentration of colostrum decreases with time after parturition, even if the cow is not milked (Kruse, 1970a).

Edwards (1982) studied the behavior of 161 Friesian dairy calves during the first 6 hr postpartum. Calves failing to suckle within 6 h of birth were subsequently assisted to suckle and recorded as having a time to first suckling of > 360 min. At the time of calving, a subjective assessment of udder conformation was made. Udder depth was scored on a 3-point scale with a score of 1 denoting a small udder, with teats more than 50 mm above the hock; a score of 2 denoting an udder with the teats at hock level; and a score of 3 denoting a pendulous udder, with the teats more than 50 mm below the hock. It was shown that calves from older cows took longer to suckle and there was a higher probability that they would not suckle during the first 6 h of life. Udder conformation was related to parity, with older cows tending to have more pendulous udders and longer, fatter teats. Calves from cows with pendulous udders spent more time teat-seeking on incorrect parts of the body of their dam before suckling, and spent more time nuzzling at the udder without successfully locating and suckling a teat. Calves from dams with an udder depth score of 1 (n = 20) had a median time to first suckling of 128 min, whereas those from dams with an udder depth score of 2 (n = 79) had a median time of 209 min, and those from dams with an udder depth score of 3 (n = 34) had a median time of 320 min.

Selman et al. (1970) observed the behavior of 30 calves during the first 8 h postpartum. Dams were categorized as beef cows, dairy heifers, or dairy cows. They were then sub-categorized into those with well-formed (i.e. optimal shape for suckling) and poor-formed udders according to conformation prior to parturition. Calves from cows with well-formed and poor-formed udder shape were compared for mean first suckling time. Calves born to poor-formed dams spent significantly longer at teat seeking than those calves born to well-formed dams. Cows with well-formed udders had a mean first suckling time of 17.1 min while those with poor-formed udders had a mean time of 39.6 min. The time from birth to the time of the calf's first suckle was lower for calves from cows with well-formed udders (79.4 min) than those calves born from cows with characteristically poor-formed udders (220.1 min). The measurement taken for first suckling began when the calves first stood after parturition and ended when the calves first found the teats of the cows and began to suck. Beef cows had a lower first suckle time than the other two groups; this is possibly due to the more pendulous udders of dairy cows, which make the teats more difficult for calves to find.

Riley et al. (2001) studied 116 F_1 cows in central Texas. These cows were sired by Angus, Gray Brahman, Gir, Indu-Brazil, Nellore, and Red Brahman bulls from Hereford dams. The authors concluded that size, overall condition, and health of the cow's udder had a great effect on the survival and growth characteristics of her progeny. Cows sired by Nellore had smaller postpartum teat length, and larger udder support scores (1 to 9 scale; 1 being very loose and pendulous and 9 being very tight) than Gir, Indu-Brazil, and Red Brahman crossbreds, and smaller postpartum teat length than all five other crossbred groups. Angus crossbreds had the greatest udder support scores in the youngest three age categories; however, udder support scores for these cows decreased the most of all breed groups as cows aged. Nellore sired cows tended to have the highest udder support scores, most notably as 7-yr-old cows and older. Angus had the smallest increase in teat diameter with age. At the time of report, Nellore crossbred cows had the highest percentage (60%) of cows remaining in the herd. The authors partially attributed this to an overall more desirable udder condition. Udder-related problems led to the removal of 22.4% of the 116 cows evaluated. This was the second highest cause for removal in the study behind reproductive failure (i.e. failing to wean a calf for the second time). Culling factors attributed to udder conformation included structural problems (e.g. excessively large teats, injured or diseased udders), inadequate milk production, or combinations of these factors. The study noted few breed differences for teat distension, teat placement, teat shape, and udder balance. Results from analyses of teat diameter, teat length, and udder support scores seemed to best describe breed differences.

Any deviation from correct teat and udder conformation results in a sharp decrease in nursing ability in purebred gray Brahman cattle (Wythe, 1970). Riley et al. (2004) stated that the structure and quality of the dam's udder was potentially one of the most important age-dependent factors affecting calf mortality. The notes associated with calving records reported that in 41 of the 392 calf deaths and in 46 of the 378 calves with poor vigor, the cows were reported as having poor udders or teats.

MacNeil and Mott (2006) studied the Line 1 Hereford population maintained by the USDA-ARS at Miles City, MT. Observations of udder score (n = 1,686) from 622 cows were analyzed to partition udder scores of cows into genetic and nongenetic components. Mean estimate of the phenotypic variance of udder score was 1.89 (1 to 9 scale, with 1 being very pendulous with balloon teats and 9 being an ideal mammary system) and the estimates of phenotypic correlations between preweaning gain and udder score, and milk production and udder score were –0.07 ± 0.04 and –0.09 ± 0.05, respectively. Genetic correlation of milk production with udder score was estimated as –0.36 ± 0.16. The authors concluded that an undesirable consequence of selection to increase milk production is the degradation of udder quality, if not offset by simultaneous selection for udder conformation.

Fuerst-Waltl et al. (1988**)** reported heritability for udder depth, fore udder attachment, and rear udder height as 0.31, 0.26, and 0.22, respectively. MacNeil and Mott (2006) reported a similar heritability for udder score in Hereford cattle as 0.23. Schrooten et al. (2001) also reported heritability for udder depth, fore udder attachment, and rear udder height with values of 0.45, 0.35, and 0.35, respectively. Bradford et al. (2015) reported an estimated heritability of 0.32 for udder suspension.

Riley et al. (2001) stated there may not be actual benefit from increased milk yield in range conditions if it is difficult for a calf to nurse for any reason. Work from Van Vleck and Norman (1972) and Chyr (1974) both indicated that cows with shallow udders milk less, but udders that are too deep tend to have more mastitis (Young et al. 1960). An intermediate udder depth appears to be preferable in the dairy industry. Unfavorable genetic correlations between milk performance traits and functional traits have been reported (Simianer et al., 1991; Castillo-Juárez et al., 2000). Cole et al. (2011) said that because an intermediate value may be optimal for many conformation traits, very few SNPs were driven towards fixation, thus intermediate frequencies should allow considerable flexibility in genetic selection for improving conformation traits and associated functionality.

Teat Diameter

Dairy cow studies often report the relationship between larger teat diameter and increased milk production (Moore et al. 1981; Seykora and McDaniel, 1986). However, large teat diameter has also been linked to increased mastitis in dairy cows (Hickman, 1964; Seykora and McDaniel, 1986). Larger teat diameter corresponds with larger teat canals, allowing for an increased rate of milk flow, but also a larger passageway for irritants or bacteria to enter the mammary system.

Frisch (1982) studied the effect bottle teats have on calf pre-weaning growth and weaning weight in Queensland, Australia. Bottle teats are those with a diameter greater than or equal to 35 mm. The classification does not refer in any way to teat shape. The study measured the teat length and diameter of 892 cows from 8 different breeding lines within 2 days of calving. Lines of cattle used were grade Brahman and Africander, F_4 and later generations of Hereford × Shorthorn (HS), Brahman × HS and Africander ×

HS, and F_1 and F_2 generations of Africander-HS × Brahman-HS. The grade Brahman and Africander cows were from ¾ to purebred *Bos indicus* cattle. It is important to note that, at the time, Africander was considered a *Bos indicus* breed in Australia. The breed has since been described as Sanga type within the *Bos taurus* classification. Data were also collected from commercial lines of Herefords and F_1 Sahiwal × Hereford. Frisch (1982) found that incidence of bottle teats was higher in cows aged 5 yr or more than in young cows. Mortality rates became unacceptably high at this level. Calves born to cows with 4 bottle teats had disproportionately high mortality rates. Cows without any bottle teats had the lightest calves at weaning and cows with 4 bottle teats had the heaviest calves at 2 months of age. This is assumed to be because of the association of large teat diameter with increased milk production. However, in beef cattle**,** the significance of increased milk production is decreased if the calf is unable to nurse as a result of large teat diameter, particularly in the critical hours postpartum. Frisch (1982) found that bottle teats were the most important single cause of calf mortality from birth to 2 months of age. Calves born alive to cows with 4 bottle teats suffered 0.486 mortality. A high proportion of these calves could not suck from bottle teats and this eventually led to their deaths. Cows with 1 to 3 bottle teats had a calf mortality rate of 0.070 to 0.076. 7 cows had both 4 bottle teats and pendulous udders. None of the calves born to these cows could suck, and all seven were dead within a few days of birth (Frisch, 1982).

Chrystal et al. (1999) measured teat diameters on 1,740 Holstein cows with 2,261 lactations in nine herds. Reported estimate of heritability for teat diameter was 0.23, 0.27, and 0.35 for 1st, 2nd, and all lactations combined, respectively. Bradford et al.

(2015) estimated genetic parameters for udder traits in Hereford cattle and reported a heritability estimate of 0.28 for teat size.

Teat Length

Short et al. (1991) reported high genetic correlations between teat length and other udder size traits in Holstein cows. The authors found longer teats to be associated with weaker udder support and deeper udders. Short teats are easier for calves to reach and are usually easier to nurse. In the previously discussed Frisch (1982) study, teat diameter was determined to be a more important factor than teat length in the ability of the calf to nurse, though an optimum range was established for teat length. Calves born to cows with all 4 teats 50 mm or less in length averaged 139 kg, 5 kg less than calves from cows with at least one teat longer than 50 mm. Calves from cows with at least one teat longer than 90 mm had a mortality rate of 0.230, which was significantly higher than the rate for calves born to cows with 4 teats shorter than 90 mm (0.078) (Frisch, 1982). According to Selman et al. (1970), if teats were too long or too large, calves had difficulty getting the ends into their mouths and several calves spent a long time mouthing and nibbling the teats that were too large. This is especially a problem in beef cow-calf operations where cattle are typically maintained within an extensive management operation and assistance to calves is not always readily available.

Donald (1960), Chyr (1973), and Freeman (1976) reported that cows with shorter teat length had higher milk production than cows with long teat lengths. Conversely, Rogers and Hargrove (1993) reported a correlation between shorter teat length and lower milk production. In addition, Rogers and Hargrove (1993) also reported Holsteins with shorter teats were associated with lower somatic cell milk counts than long teats, and seemingly less incidence of mastitis. It should be noted that, traditionally, larger dimensions of the udder and teats correspond with higher milk production. Dairy studies may often report findings based on machine milking, where shorter teats are more amenable to the equipment used, therefore providing results that differ from traditional beef cattle production views.

Fuerst-Waltl et al. (1988**)** and Schrooten et al. (2001) reported heritabilities for teat length as 0.25 and 0.45, respectively.

Marker Discoveries and Location

According to Cole et al. (2009), genetic effects must exist somewhere on the chromosomes for any trait with a nonzero heritability, and marker locations and estimates of effects can be used to identify chromosomal segments of interest for a functional genomic study. A major objective of QTL studies is to find genes or markers that can be implemented in breeding programs via marker assisted selection (Khatkar et al., 2004). Furthermore, functional traits, or those characteristics of an animal that increase the efficiency by reducing costs of input (Groen et al., 1997), have low heritabilities that hinder the progress of conventional breeding designs for functional traits. Marker assisted selection may aid in improving this situation (Kuhn et al., 2003).

Association analyses for udder traits have apparently not been done in beef cattle, therefore, subsequent reports of markers and chromosomal locations were collected entirely from dairy cattle and sheep studies. Ashwell et al. (2001) selected semen samples from the Dairy Bull DNA Repository Marker from eight large US Holstein families. QTL were studied by using the granddaughter design. Marker findings are reported in Table 1. Cole et al. (2009) examined genotypes for 38,416 SNP scored in 5,360 Holstein bulls. Putative QTL for fore udder attachment and udder depth on BTA 5 were reported at 94.8 Mb. This SNP is bracketed by microsatellites associated with udder depth at 90.8 and 109 Mb reported by Schrooten et al. (2000) and Ashwell et al. (2004), respectively. Kolbehdari et al. (2008) genotyped 462 Canadian Holstein bulls and identified SNP rs41636734 at 53,743,293 bp on BTA 18 to be associated with conformation and the mammary system.

Table 1. Previously reported markers with detected association with udder traits

Trait	BTA or OAR	Location	Reference
Front teat placement[3]	1	119 cM	Ashwell et al. (2005)[1]
	5	119 cM	
	6	0 cM	
		N/A	Cole et al. (2011)[1]
	10	44 cM	Schrooten et al. (2000)[1]
	14	48 cM	Ashwell et al. (2005)[1]
	15	52 cM	
	19	67 cM	Schrooten et al. (2000)[1]
	23	24 cM	Ashwell et al. (2005)[1]
	26	42 cM	
	29	21 cM	
Fore udder attachment[4]	2	2 cM	

BTA = *Bos taurus*

OAR = *Ovis aries*

[1] Study conducted on Holstein or Holstein-Friesian cattle

[3] Teat placement measurements range from extremely wide placement on outside of quarter to base of teats on extreme inside of quarter (Holstein Association USA, Inc., 2014).

[4] Fore udder attachment is the evaluation of the strength of the fore udder attachment to the body wall by the lateral ligaments (Stamschror et al., 2000). Measurements range from extremely loose to extremely snug and strong (Holstein Association USA, Inc., 2014).

Table 1. Continued

Trait	BTA or OAR	Location	Reference
Fore udder attachment[4]	5	112 cM	Ashwell et al. (2005)[1]
	10	51 cM	Schrooten et al. (2000)[1]
		116 cM	Ashwell et al. (2005)[1]
		N/A	Cole et al. (2011)[1]
	13	0 cM	Schrooten et al. (2000)[1]
		23 cM	
		63 cM	Ashwell et al. (2005)[1]
	14	N/A	Ashwell et al. (2001)[1]
	15	36 cM	Ashwell et al. (2005)[1]
	18	33 cM	Ashwell et al. (2005)[1]
	19	68 cM	Schrooten et al. (2000)[1]
	20	45.5 cM	Ashwell et al. (2001)[1]
		66 cM	Ashwell et al. (2005)[1]
	23	16 cM	
	24	48 cM	
	25	62 cM	Schrooten et al. (2000)[1]
	26	3 cM	
	28	8 cM	Ashwell et al. (2005)[1]
	29	23 cM	
Mammary system	18	53.7 Mb	Kolbehdari et al. (2008)[1]
Rear teat placement[3]	2	N/A	Cole et al. (2011)[1]
Rear udder height[5]	2	N/A	Cole et al. (2011)[1]
	5	88 cM	Schrooten et al. (2000)[1]
	10	N/A	Cole et al. (2011)[1]
	11	N/A	
	13	66 cM	Ashwell et al. (2005)[1]
	17	69 cM	
	18	28 cM	

BTA = *Bos taurus*
OAR = *Ovis aries*
[1] Study conducted on Holstein or Holstein-Friesian cattle
[3] Teat placement measurements range from extremely wide placement on outside of quarter to base of teats on extreme inside of quarter (Holstein Association USA, Inc., 2014).
[4] Fore udder attachment is the evaluation of the strength of the fore udder attachment to the body wall by the lateral ligaments (Stamschror et al., 2000). Measurements range from extremely loose to extremely snug and strong (Holstein Association USA, Inc., 2014).
[5] Rear udder height is the distance between the bottom of the vulva and top of the milk secreting tissue (Stamschror et al., 2000). Measurements range from extremely low to extremely high (Holstein Association USA, Inc., 2014).

Table 1. Continued

Trait	BTA or OAR	Location	Reference
Rear udder height[5]	28	25 cM	Ashwell et al. (2005)[1]
	29	16 cM	
Rear udder width[6]	28	16 cM	
Somatic cell score[7]	5	N/A	Heyen et al. (1999)[1]
	7	N/A	
Teat length[8]	5	43 cM	Ashwell et al. (2005)[1]
	6	133 cM	
	11	N/A	Cole et al. (2011)[1]
	16	48 cM	Ashwell et al. (2005)[1]
	17	78 cM	
		N/A	Cole et al. (2011)[1]
	18	N/A	Schnabel et al. (2005)[1]
	19	76 cM	Ashwell et al. (2005)[1]
	21	N/A	Schnabel et al. (2005)[1]
	26	31 cM	Ashwell et al. (2005)[1]
Teat placement[9]	OAR 7	17 cM	Gutiérrez-Gil et al. (2008)[2]
Udder[10]	25	80 cM	Schrooten et al. (2000)[1]
	26	0 cM	
Udder attachment[11]	13	30.3 cM	Lund et al. (2008)[1]
	26	53 cM	
	OAR 26	74 cM	Gutiérrez-Gil et al. (2008)[2]

BTA = *Bos taurus*

OAR = *Ovis aries*

[1] Study conducted on Holstein or Holstein-Friesian cattle

[2] Study conducted on Spanish Churra dairy sheep

[6] Rear udder width is evaluated at the point of attachment (Stamschror et al., 2000). Measurements range from narrow rear udder to extremely wide rear udder (Holstein Association USA, Inc., 2014).

[7] Somatic cell score ranges from 0 to 9; Lower somatic cell scores reflect more resistance to mastitis. (Van Dormaal, 2007)

[8] Teat length measurements range from 1.25 in or smaller to 3.25 in or longer (Holstein Association USA, Inc., 2014).

[9] Teat placement measurements range from extremely wide placement on outside of quarter to base of teats on extreme inside of quarter (Holstein Association USA, Inc., 2014).

[10] Udder, as it relates to the dairy cattle scorecard, includes udder depth, rear udder, teat placement, udder cleft, fore udder, teats, and udder balance and texture (Stamschror et al., 2000; Schrooten et al., 2001).

[11] Udder attachment measurements range from extremely loose to extremely snug and strong (Holstein Association USA, Inc., 2014).

Table 1. Continued

Trait	BTA or OAR	Location	Reference
Udder cleft[12]	1	119 cM	Ashwell et al. (2005)[1]
	7	8 cM	
		N/A	Cole et al. (2011)[1]
	14	51 cM	Ashwell et al. (2005)[1]
	15	55 cM	
	22	0 cM	
	27	N/A	Cole et al. (2011)[1]
Udder depth[13]	4	73.7 cM	Lund et al. (2008)[1]
	5	109 cM	Schrooten et al. (2000)[1]
	13	72 cM	Ashwell et al. (2005)[1]
	15	37 cM	
	16	61 cM	Ashwell et al. (2001)[1]
	18	36 cM	Ashwell et al. (2005)[1]
	19	34 cM	Schrooten et al. (2000)[1]
	20	59.1 cM	Lund et al. (2008)[1]
	22	52.1	
	23	49 cM	Ashwell et al. (2005)[1]
		59.1 cM	Lund et al. (2008)[1]
	24	56 cM	Ashwell et al. (2005)[1]
	26	66 cM	
		N/A	Cole et al. (2011)[1]
	27	44 cM	Schrooten et al. (2000)[1]
	29	37.2 cM	Lund et al. (2008)[1]
	OAR 20	8 cM	Gutiérrez-Gil et al. (2008)[2]
Udder shape	OAR 15	1 cM	

BTA = *Bos taurus*

OAR = *Ovis aries*

[1] Study conducted on Holstein or Holstein-Friesian cattle

[2] Study conducted on Spanish Churra dairy sheep

[12] Udder cleft: a deep udder cleft is an indicator of a strong median suspensory ligament. This is an elastic ligament, up the center of the udder, that provides 60% of the udder's support (Stamschror et al., 2000). Measurements range from weak cleft to extremely strong cleft (Holstein Association USA, Inc., 2014).

[13] Udder depth is evaluated as the relationship of the udder floor relative to the hocks (Stamschror et al., 2000). Measurements range from very deep udder floor well below hocks to extreme height of udder floor above hocks (Holstein Association USA, Inc., 2014).

In a study involving contemporary U.S. Holstein cows, Cole et al. (2011) identified 3,100 effects of 1,586 SNPs. There were a large number of additive SNP effects that reached 5% genome-wide significance for each trait, therefore only the top 100 effects for each trait were reported. The majority of the 1,586 SNPs each affected one trait, whereas 27 SNPs each affected 10 or more traits. The effects were unevenly distributed over all 29 BTA and the X chromosome. Some chromosomes had large numbers of SNP effects, and different chromosomes were commonly associated with different traits. The authors found that chromosomes with a large number of effects for a trait did not necessarily give the most significant effect associated with that trait. Traditional predicted transmitting abilities for each trait calculated by the U.S. Department of Agriculture were the analyzed dependent variables for association with SNPs. Cole et al (2011) reported the 100 most significant SNP effects for predicted transmitting abilities of various traits. Significance levels varied by trait.

Schrooten et al. (2000) collected semen samples and extracted DNA from 949 young Holstein Friesian bulls belonging to 22 half-sib families. The number of young bulls per grandsire ranged from 12 to 147 and had an average family size of 42. Genotypes for 277 microsatellite markers covering 29 autosomes were determined for all young bulls and grandsires. The authors found the genetic correlation between fore udder attachment and udder depth estimated from female progeny of these bulls to be around 0.70.

Gutiérrez-Gil et al. (2008) conducted a genome scan to detect QTL influencing udder morphology traits in Spanish Churra dairy sheep. Data were collected from 739 ewes from 11 half-sib families and included the following phenotypic traits: udder depth, udder attachment, teat placement, teat size, and udder shape. The average family size was 67 ewes/ram. The authors cite Casu et al. (2003) as stating functional traits related to udder morphology have a substantial effect on a dairy sheep's lifetime. This statement corroborates similar findings in cattle studies. Table 2 shows the putative correspondence between the QTL reported by Gutiérrez-Gil et al. (2008) and similar QTL reported in dairy cows.

Marker findings for Schrooten et al. (2000), Ashwell et al. (2001), Gutiérrez-Gil et al. (2008), Kolbehdari et al. (2008), and Cole et al. (2011), as well as findings from Heyen et al. (1999), Ashwell et al. (2004), Ashwell et al. (2005), Schnabel et al. (2005), and Lund et al. (2008) are outlined in Table 1. All of these studies involved Holstein or Holstein-Friesian cattle except Gutiérrez-Gil et al. (2008) which invloved Spanish Churra dairy sheep.

As stated by Garrick (2011), different countries sometimes define traits in different ways, and have different harvest end points, resulting in imperfect relationships between traits in different countries. As a result, there has been a lack of agreement in the QTL mapping community on how to analyze data from QTL studies and what significance thresholds should be used to detect and report QTL. Consequently, it is not surprising that results from these studies sometimes confirmed the same QTL and, in other cases, provided conflicting results (Ashwell et al., 2001).

Table 2. Putative correspondence between QTL found in Churra Sheep and other similar QTL reported in dairy cows [Adapted from Gutiérrez-Gil et al. (2008).]

QTL detected in Churra Sheep				**QTL detected in the orthologous region of cow**				
Chromosome	Trait	Location	Flanking Markers	Chromosome	Trait	Location	Flanking Markers	Reference
OAR7	Teat placement[1]	17 cM	BM3033-BMS528	BTA 10	Front teat placement[1]	44 cM	BRRIBOold-BMS861	Schrooten et al. (2000)
					Fore udder attachment[2]	51 cM	TGLA378	Schrooten et al. (2000)
OAR15	Udder shape	1 cM	MCMA16-BR3510	BTA 15	Udder depth[3]	37 cM	BMS2684-HBB	Ashwell et al. (2005)
					Udder cleft[4]	55 cM	HBB-ILSTS061	Ashwell et al. (2005)
OAR20	Udder depth[3]	8 cM	INRA132-DYA	BTA 23	Front teat placement[1]	24 cM	BM1258-MGTG7	Ashwell et al. (2005)
					Fore udder attachment[2]	16 cM	CSSM5-BM1258	Schrooten et al. (2000)
OAR26	Udder attachment[2]	74 cM	CSSM43-BM203	BTA 27	Udder depth[3]	44 cM	HUJI-13	Schrooten et al. (2000)

BTA = *Bos taurus*
OAR = *Ovis aries*

[1] Teat placement measurements range from extremely wide placement on outside of quarter to base of teats on extreme inside of quarter (Holstein Association USA, Inc., 2014).

[2] Fore udder attachment measurements range from extremely loose to extremely snug and strong (Holstein Association USA, Inc., 2014).

[3] Udder depth measurements range from very deep udder floor well below hocks to extreme height of udder floor above hocks (Holstein Association USA, Inc., 2014).

[4] Udder cleft measurements range from weak cleft to extremely strong cleft (Holstein Association USA, Inc., 2014).

MATERIALS AND METHODS

All procedures involving animals were approved by the Texas A&M University Institutional Animal Care and Use Committee. Data were collected from cows at the Texas A&M University Agricultural Research Center at McGregor in the McGregor Genomics Project. The cows in this study were born in both spring and fall of 2003 through 2006 and the spring 2007 seasons.

Cows were sired by 4 different F_1 Nellore x Angus bulls and belonged to one of 17 different project families. The dams of those cows in project family numbers 70 to 77 and 80 to 84 were F_1 Nellore x Angus. Cows in these families were produced by embryo transfer (ET) and are full siblings to others in their family. Cows in project families 95 to 98 were all out of either half Brahman and half Angus, or half Brahman and half Hereford F_1 or F_2 dams. . Cows in project families 95 to 98 were produced via natural service (NS) from the same 4 sires as the ET cows and are half siblings to others in their respective families. Females were born at McGregor, with the exception of 10 of the 2003-born cows. These females came from the Texas A&M Agricultural Research Center at Angleton. Heifers born in the spring and fall were exposed to bulls in order to calve at the age of 2. All cows were bred to Angus bulls for their first calf. Any heifers that did not conceive and calve at age 2 were added to the spring calving herds and were subsequently managed in order to calve in the spring at 2.5 years of age. Nine cows calved at 2 years of age in the fall: one in 2003, two in 2004, two in 2005, and four calved in 2006. From the 2004 to 2008 breeding season the cows were pastured in groups according to age and managed similarly. Heifers were managed in separate pastures from cows in each year.

Cows were kept on a variety of warm season pastures including coastal bermudagrass (*Cynodon dactylon*), Eastern gamagrass (*Tripsacum dactyloides*), Kleingrass (*Panicum coloratum*), and native vegetation pastures. Supplementation with mineral and salt was provided in the spring and summer. Coastal bermudagrass hay or sudan grass (*Sorghum sudanese*) hybrid hay was supplemented in the winter.

Cows were evaluated for several traits including udder support scores, teat diameter and length within 24 h of calving at the time of birth weight collection. These traits were subjectively evaluated by trained TAMU personnel at the McGregor Research Center. Udder support scores ranged from 1 to 9 with 1 being very loose and pendulous and 9 being very tight. Udder support relates to the degree and strength of the front and rear udder attachment.

Teat diameters and lengths were individually recorded for each quarter from a single evaluator as subjective estimates. The diameter and length were estimated to the nearest $^1/_8$ of an inch. Teat length was estimated between the upper and lower extremity of the teat. Teat diameter was recorded at the midpoint of the teat.

Blood samples were previously collected on all live-born animals in the population, and the BovineSNP50 assay (Illumina Inc., San Diego, CA) was used to obtain genotypes on 54,001 markers for all animals. For quality purposes, if a single SNP marker had less than 90% of the animals successfully genotyped, had a minor allele frequency less than 0.05, and/or were out of Hardy-Weinberg equilibrium proportions, the marker was not included in future analyses. This left 34,980 SNP for use in this study.

Statistical Analyses

Mixed linear model procedures of SAS (SAS Institute Inc., Cary, NC) were used to evaluate the fixed effect of cow age (distinct categories for 2, 3, 4, and ≥ 5 years of age) nested within birth year-season combinations. Cow was included as a random effect. Repeated measures analyses were conducted to generate residuals for each cow. These residuals were averaged for each cow (n = 295) to get a single value for each trait to generate a phenotypic residual file.

Association analyses for teat diameter and length on each quarter, the average of the four quarters, and udder support score were conducted using the Q-K procedures (Yu et al., 2006) of JMP Genomics (SAS Inst., Inc., Cary, NC) with fixed effects identified from the preliminary analyses and regression of trait on genotypic values, which were coded as 0 for the homozygote of the allele with the highest frequency and 2 assigned to the other homozygote, with heterozygotes assigned values of 1. Using methodology of Benjamini and Hochberg (1995), the false discovery rate was constrained to 0.05. The genetic covariance among animals in this project was modeled by constructing the genomic relationship matrix based upon genotypes from the Bovine SNP50 using procedures of Yu et al. (2006). There were 776 individuals in the pedigree.

Locations of the genomic feature closest to each SNP locus associated for each trait were determined using R statistical software and the package Map2NCBI (Hanna and Riley, 2014). Map coordinates reported are from the bovine UMD_3.1 build (Zimin et al., 2009).

Additionally, cows were evaluated to determine family effects for udder support, average teat diameter, and average teat length using the MIXED procedure (SAS Inst. Inc., Cary, NC).

RESULTS AND DISCUSSION

Preliminary analyses indicated that cow age nested within birth year-season combinations was an efficient parameterization of these effects ($P < 0.001$). Gladney (2008) reported udder and teat characteristics from cows in this study through the spring 2007 calving season. Cooper (2011) analyzed these data through the 2009 records. Simple means for traits in the analyses are shown in Tables 3 and 4 for all cows and cows by age, respectively.

Evaluation of Family Structure

Least squares means for udder support, average teat diameter, and average teat length are presented in Table 5. Family was significant ($P < 0.05$) across all three traits (udder support: $P = 0.0339$; average teat diameter: $P = 0.0003$; average teat length: $P < 0.001$). Individual teat traits were not evaluated.

Families 73 (7.2) and 82 (7.0), which were both sired by 432H, had the highest ($P < 0.001$) scores for udder support, and family 95 (5.7), sired by 297J, had the lowest ($P < 0.001$) scores.

Family 82 (1.5 cm) had the smallest ($P = 0.003$) average teat diameter, and family 95 (3.3 cm) had the largest ($P < 0.001$) diameter.

Families 82 (2.5 cm) and 72 (3.6 cm), both sired by 432H, had the shortest ($P < 0.001$) average teat length, and families 84 (5.6 cm), 76 (5.6 cm), and 98 (5.4 cm), all sired by 551G, had the longest ($P < 0.001$).

Marker Associations

There were 15 and 1 detected SNP associations (-log[FDR] ≥ 1.3) for udder support and average teat diameter, respectively (Table 6).

Table 3. Simple means of udder support, average teat diameter (cm), and average teat length (cm) for all cows

Trait	N	Mean	SD	Minimum	Maximum
Udder support score[1]	1,746	5.89	1.11	2	9
Average teat diameter	1,744	2.78	1.43	0.48	12.86
Average teat length	1,744	4.9	1.8	1.51	11.91

[1] Udder support was scored on a 1 to 9 scale with 1 being very loose and pendulous and 9 being very tight.

Table 4. Simple means of udder support, average teat diameter (cm), and average teat length (cm) for cows by age (yr)

Trait	Cow Age	N	Mean	SD	Minimum	Maximum
Udder support score[1]	2	192	6.83	0.57	5	9
	3	245	6.61	0.62	4	8
	4	246	6.29	0.85	3	7
	5+	1,063	5.46	1.1	2	7
Average teat diameter	2	193	1.8	0.31	1.27	3.49
	3	244	2.1	0.62	1.59	5.4
	4	246	2.44	0.95	1.59	7.3
	5+	1,061	3.2	1.6	0.48	12.86
Average teat length	2	193	3.19	0.89	1.51	6.67
	3	244	3.86	1.32	1.67	8.73
	4	246	4.57	1.47	1.91	9.21
	5+	1,061	5.52	1.77	1.59	11.91

[1] Udder support was scored on a 1 to 9 scale with 1 being very loose and pendulous and 9 being very tight.

Table 5. Means and SE of udder support, average teat diameter (cm), and average teat length (cm) for cow families

Family[1]	Sire of family	Dam of family	Udder support score[2]	Average teat diameter	Average teat length
70	297J	431H	6.1 ± 0.30	2.5 ± 0.25	4.5 ± 0.29
71	297J	760H	6.1 ± 0.28	2.6 ± 0.18	5.0 ± 0.22
72	432H	511G	6.4 ± 0.26	2.2 ± 0.22	3.6 ± 0.26
73	432H	732H	7.2 ± 0.50	2.0 ± 0.66	4.1 ± 0.81
74	437J	640H	6.4 ± 0.39	2.0 ± 0.47	4.3 ± 0.57
75	437J	728H	6.6 ± 0.27	2.2 ± 0.22	3.8 ± 0.27
76	551G	664J	6.7 ± 0.57	2.8 ± 0.75	5.6 ± 0.88
77	551G	787G	6.1 ± 0.27	2.5 ± 0.23	4.2 ± 0.27
80	551G	429H	6.1 ± 0.28	2.4 ± 0.21	4.4 ± 0.26
81	437J	636H	6.3 ± 0.26	2.3 ± 0.22	4.1 ± 0.26
82	432H	559J	7.0 ± 0.35	1.5 ± 0.40	2.5 ± 0.49
83	437J	637H	6.6 ± 0.27	2.0 ± 0.23	4.2 ± 0.28
84	551G	911H	6.4 ± 0.30	3.0 ± 0.26	5.6 ± 0.31
95	297J		5.7 ± 0.28	3.3 ± 0.20	5.1 ± 0.24
96	432H		6.4 ± 0.24	2.2 ± 0.16	3.7 ± 0.19
97	437J		6.3 ± 0.26	2.5 ± 0.23	4.1 ± 0.28
98	551G		5.8 ± 0.34	2.5 ± 0.35	5.4 ± 0.43

[1] Cows were sired by 4 different F_1 Nellore-Angus bulls and belonged to one of 13 different project families. Dams of those cows in project family numbers 70 to 77 and 80 to 84 were F_1 Nellore-Angus. Cows in these families were produced by embryo transfer and are full siblings to others in their family. Cows in project families 95 to 98 were all out of either half Brahman and half Angus, or half Brahman and half Hereford dams and were produced via natural service from the same 4 sires as the embryo transfer cows and are half siblings to others in their families.

[2] Udder support was scored on a 1 to 9 scale with 1 being very loose and pendulous and 9 being very tight. Udder support relates to the degree and strength of the front and rear udder attachment.

Table 6. Genome-wide significant SNP for udder support on BTA 5

SNP name	Position (Mb)	Nearest gene	Distance[1] (bp)
ARS-BFGL-NGS-32842	21.6	*SPCS3*	98926
ARS-BFGL-NGS-35234	22.7	*LOC100848544*	56441
ARS-USMARC-637	32.6	*VDR*	within
Hapmap58789-rs29016364	39	*LOC785294*	104837
ARS-BFGL-NGS-29758	39.9	*LOC100139418*	11249
Hapmap58370-rs29014396	43	*PTPRR*	within
BTA-112529-no-rs	43.6	*LOC786074*	59452
BTB-00227037	43.7	*C5H12orf28*	9882
ARS-USMARC-614	45.7	*IL22*	within
ARS-BFGL-NGS-22009	46.3	*DYRK2*	39671
Hapmap24085-BTA-143102	46.4	*DYRK2*	30159
ARS-BFGL-NGS-98210	46.5	*DYRK2*	119742
ARS-BFGL-NGS-114616	46.5	*DYRK2*	163137
ARS-BFGL-NGS-76882	46.5	*DYRK2*	192136
BTA-11044-rs29016809[2]	48.1	*LOC100848387*	45909

[1] Reflects distance, in bp, of associated SNP from the nearest gene.

[2] This marker was also detected in the average teat diameter analysis.

A total of 15 significant SNPs, exclusively on BTA 5, were detected for udder support (Figure 1). Of these SNPs, three were located within genes; *VDR*, *IL22,* and *PTPRR.* The other 12 associated SNPs were located at distances of 9,882 to 192,136 bp from the nearest known genes. Another gene, dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 (*DYRK2*), did not have any markers located within its region, but 5 markers were located nearby (30,159 to 192,136 bp).

A single SNP on BTA 5 was found to be associated with average teat diameter (Figure 2). This SNP was found 45,909 bp from *LOC100848387,* which encodes 60S ribosomal protein L7. This SNP also had the strongest association with udder support.

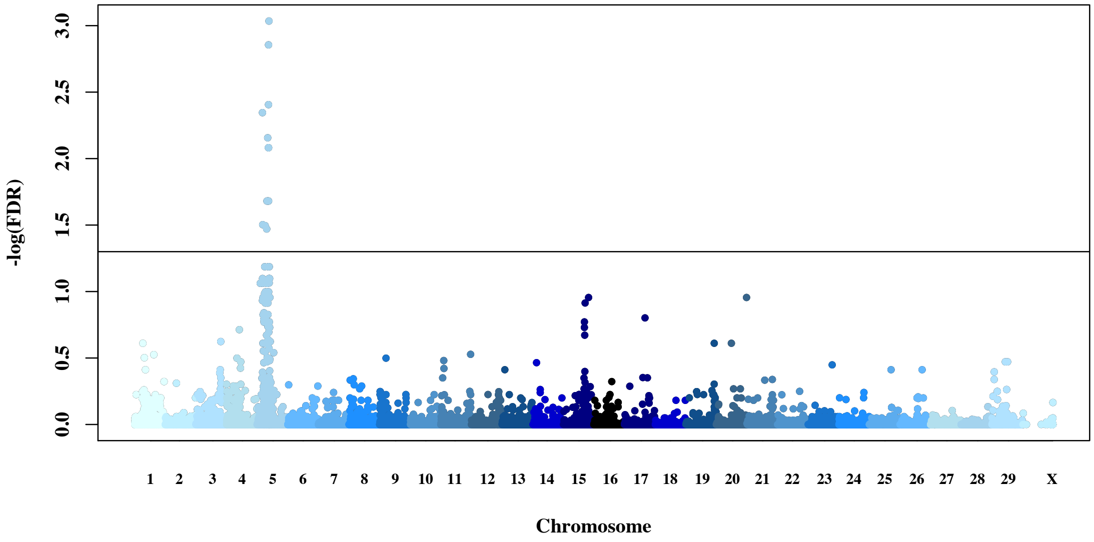

Figure 1. Udder support-SNP marker associations by significance (-log[FDR] = 1.3 at horizontal line) and chromosome.

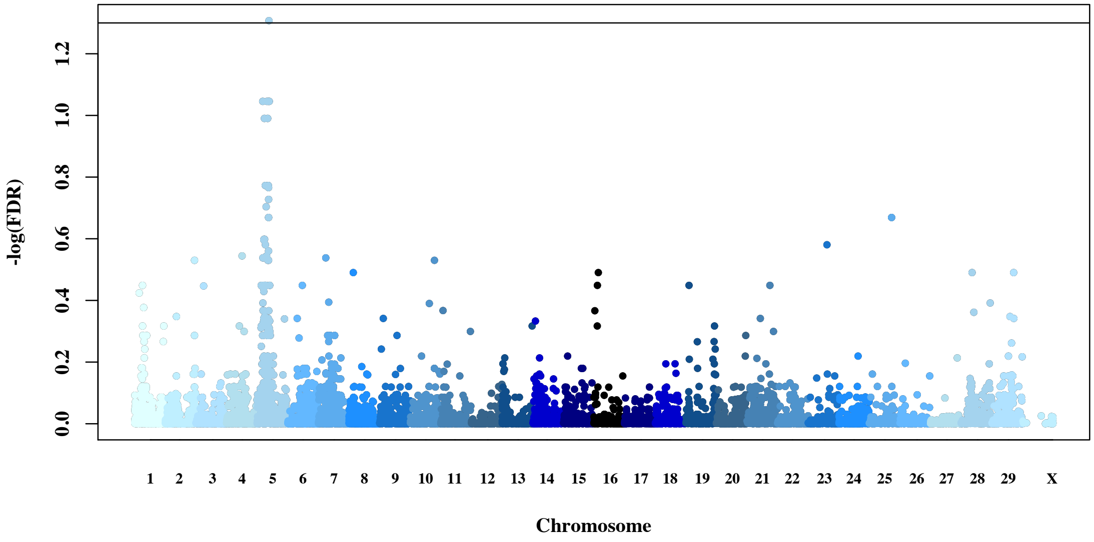

Figure 2. Average teat diameter-SNP marker associations by significance (-log[FDR] = 1.3 at horizontal line) and chromosome.

All 15 significant markers were located within a 26.4 Mb region on BTA 5 (21.6 to 48.1 Mb). Of those 15 markers, 10 were located within a 5.1 Mb region (43.0 to 48.1 Mb).

Vitamin D Receptor (VDR)

Marker-ARS-USMARC-637 (-log10[FDR] = 1.50) is within the boundaries of the *VDR* gene. This gene, which ranges from 32.5to 32.6 Mb on BTA 5, encodes nuclear hormone receptor for vitamin D3 (1, 25-dihydroxyvitamin D3), which plays a crucial role in calcium related metabolism and calcium homeostasis (Gao et al., 2013). Results from a study by Gao et al. (2013) investigating the association between the bovine *VDR* gene and growth traits suggested that the *VDR* gene could be used as a candidate gene for marker-assisted selection and management in beef breeding practice. *VDR* mediates the action of vitamin D3 by controlling the expression of hormone sensitive genes (Akutsu et al., 2001). It also has involvement in cell differentiation and proliferation, bone and cartilage development, and regulation of osteoporosis, as well as a vital role in calcium homeostasis (Gao et al., 2013). The receptor regulates a variety of other metabolic pathways, such as those involved in the immune response and cancer (Haussler et al., 1998). It has been suggested that maintaining vitamin D status above that needed for normal calcium homeostasis is required for optimal immune responses (Shahmohammadi et al., 2014). It is known that intra-mammary infections activate bovine macrophages found in the milk through the Toll-like Receptors pathways resulting in the up-regulation of the expression of the 1α-hydroxylase gene (Shahmohammadi et al., 2014). The expression of 1α-hydroxylase is responsible for the conversion of 25-dihydroxyvitamin D3 to active hormone 1, 25-dihydroxyvitamin D3 (Nelson et al., 2010b). According to Nelson et al. (2010a), the production of 1, 25-dihydroxyvitamin D3 leads to changes in gene expression in macrophages isolated from milk of an infected gland.

Observations indicate that the vitamin D pathway influences innate and adaptive immune responses in cattle as it does in humans. Research examining the effects of vitamin D on the severity of experimentally induced mastitis in dairy cattle has provided *in vivo* evidence for an intracrine mechanism of vitamin D signaling pathway in macrophages, and has demonstrated the potential for vitamin D to reduce the severity of bacterial infection of the bovine mammary gland (Nelson et al., 2012). It was shown by Nelson et al. (2012) that cows receiving 25-dihydroxyvitamin D3 had significantly lower bacterial counts, reduced numbers of leukocytes in mammary secretions from infected glands, and lower body temperatures than control cows demonstrating that 25-dihydroxyvitamin D3 can limit the severity of bacterial-induced mastitis.

This is relevant to this study due to the correlations between linear type traits and somatic cell score: –0.28 for udder depth, –0.21 for front teat placement, and –0.16 for udder cleft (Schutz et al., 1993). Somatic cells are indicators of both resistance and susceptibility of cows to mastitis and can be used to monitor the level or occurrence of subclinical mastitis in herds or individual cows. Most studies have focused on somatic cell score as a measure of mastitis (Heyen et al., 1999; Schrooten et al., 2000; Kuhn et al., 2003; Schrooten et al., 2004; Ashwell et al., 2005). Heyen et al. (1999) and Lund et al. (2008) found BTA 5 to be significant for somatic cell score.

Interleukin 22 (IL22)

Interleukin 22 (IL22) is located on BTA 5 and ranges from 45.721 to 45.727 Mb. Marker-ARS-USMARC-614 (-log[FDR] = 2.08) is within the boundaries of this gene. *IL22* is a member of a group of naturally occurring proteins that mediate communication between cells. Interleukins regulate cell growth, differentiation, and motility. They are particularly important in stimulating immune responses, such as inflammation. Interleukins are a subset of a larger group of cellular messenger molecules called cytokines, which are modulators of cellular behavior (Encyclopædia Britannica, 2015). According to Tizard (2012), *IL22* appears to play an important role in maintaining barrier function on exposed body surfaces. It promotes antimicrobial immunity, inflammation, and tissue repair, which could aid in mastitis prevention and resistance, thus creating a possible link to udder traits.

Furthermore, *Stat3* (Signal transducer and activator of transcription 3) is activated by a number of cytokines, including *IL22*. Cytokines and their Stat targets have important roles in lineage commitment, survival, and death of mammary gland epithelium (Hynes and Watson, 2010). *Stat3* activity is highest on the day of parturition and during the first 6 days of mammary gland involution (Watson, 2006). Genetic studies in mice have revealed roles for Stat proteins 3, 5a and b, and 6 in the adult mammary gland during a pregnancy cycle. According to Chapman et al. (1999), *Stat3* is essential for the initiation of apoptosis and remodeling following forced weaning. In the absence of *Stat3*, involution is dramatically delayed and the reversible phase can be extended for up to 6 d (Humphreys et al., 2002). Stat proteins have also been shown to play an important role in fertility and early embryonic development (Khatib, 2009).

Protein Tyrosine Phosphatase, Receptor Type, R (PTPRR)

Marker-Hapmap58370-rs29014396 (-log[FDR] = 1.47) is within the *PTPRR* gene (42.8 to 43.1 Mb).The protein encoded by *PTPRR* is a member of the protein tyrosine phosphatase (PTP) family. These proteins are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation (The NCBI Handbook [Internet], 2002).

Results from a study on mice by Aoki et al. (1999) suggested the possible contribution of PTPs to the development, involution, and remodeling of the mammary gland and their possible inhibitory action on maintaining high expression of milk genes during lactation. This study showed that some PTPs were up-regulated during gestation, suggesting that these enzymes are involved in development of the mammary gland. These PTPs then decreased during lactation. At the involution stage after weaning, most PTPs were up-regulated and their expression returned almost to the virgin level. Forced weaning in lactating mother mice also induced up-regulation.

Protein tyrosine phosphorylation has been shown to play critical roles in regulating fundamental cellular processes such as proliferation, differentiation, and development and to be controlled by the balance of protein tyrosine kinase (PTK), such as *DYRK2*, and PTP (Aoki et al., 1999).

The mammary gland undergoes dramatic changes in morphology and function during gestation, lactation, and involution under the control of a variety of peptide and steroid hormones, growth factors, cytokines (like *IL22*) and extracellular matrix. After weaning, the mammary gland is functionally and morphologically changed, as demonstrated by drastic reduction in milk gene expression (Aoki et al., 1999). Aoki et al. (1999) suggested that PTPs are involved in involution of the mammary gland and possibly the remodeling of the tissue for the next parturition. This remodeling in preparation for the next parturition would be especially important in beef cattle operations, where producers hope to retain cows with sound udders in the herd for a number of years in order to reduce the cost of replacement heifers.

Dual-Specificity Tyrosine-(Y)-Phosphorylation Regulated Kinase 2 (DYRK2)

No associated markers were found within the 46.32 to 46.33 Mb region of *DYRK2*, but five were located 30,159 to 192,136 bp from the gene (-log[FDR] =1.68 to -log[FDR] = 2.86). *DYRK2* belongs to a family of protein kinases whose members are presumed to be involved in cellular growth and/or development. The family is defined by structural similarity of their kinase domains and their capability to autophosphorylate on tyrosine residues (The NCBI Handbook [Internet], 2002). As previously mentioned, PTKs and PTPs play critical roles in mammary epithelial cell proliferation, differentiation, and apoptosis. Gallo-Hendrikx et al. (2001) wrote that PTKs and PTPs are signaling molecules involved in all aspects of development, including proliferation, differentiation, and apoptosis and reported that the balance between PTK and PTP signaling is crucial for proper mammary gland development (Tourkine et al., 1995; Edwards et al., 1998; Chodosh et al., 2000). Mammary gland involution is characterized as mammary epithelial cell apoptosis and the destruction of both lobuloalveoli and the basement membrane (Lund et al., 1996). Normal development of the mammary gland proceeds via interactions between the epithelium and the mesenchyme that start during embryogenesis and continue during pubertal outgrowth and differentiation. The function of specific peptide growth factors that bind members of the receptor tyrosine kinase family and the cytokine receptor family (including *IL22*) are required at each stage (Hynes and Watson, 2010).

CONCLUSION

A total of 15 associated (-log10[FDR] ≥ 1.3) markers were detected in the genome-wide association analyses. Udder support had 15 significant SNP detected, exclusively on BTA 5, while average teat diameter had one associated marker, also located on BTA 5. It could be of interest in the future to include breed-of-origin genotypes to determine if additional significant SNPs are identified. The results from this study identified associated markers within genes that have an involvement in the development and regulation of the mammary system and suggest that BTA 5 is significant for udder traits in beef cattle. These genetic markers could be used in the future as part of breeding strategies to aid producers in selection for important udder traits in *Bos indicus – Bos taurus* cross cows.

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