

Genome-wide association study for buffalo mammary gland morphology

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Research Article

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Abstract

This research communication describes a genome-wide association study for Italian buffalo mammary gland morphology. Three single nucleotide polymorphisms (AX-85117983, AX-8509475 and AX-85117518) were identified to be significantly associated with buffalo anterior teat length, posterior teat length and distance between anterior and posterior teat, respectively. Two significant signals for buffalo mammary gland morphology were observed in two genomic regions on the chromosome 10, and chromosome 20. One of the regions located on the chromosome 10 has the most likely candidate genes *ACTC1* and *GJD2*, both of which have putative roles in the regulation of mammary gland development. This study provides new insights into the genetic variants of buffalo mammary gland morphology and may be beneficial for understanding of the genetic regulation of mammary growth.

Introduction

The number of secretory cells in the mammary gland is critically important to milk production. As a proxy for secretory cell numbers, the mammary size was associated with milk yield in goats, sheep and cattle, and the correlation coefficients between milk yield and the measures of mammary size (udder weight, udder volume and udder capacity) were moderate (Davis, 2017). Moreover, udder conformation traits are often cited as being critically important indicators because mammary morphology is highly associated with the length of productive life and mastitis susceptibility. Therefore, it is important to understand drivers of mammary gland morphology and their roles in the determination of productive performance in dairy livestock.

Genetic selection has increased udder size in Holstein cow and Friesian cows. Genome-wide association studies (GWAS) have facilitated identification of genes and variants associated with udder size. In Fleckvieh cattle, a total of 12 quantitative trait loci (QTL) and eight candidate genes were associated with tested variants (such as udder depth and fore udder length), and among the candidate genes, *SP5* and *ADAM12* may participate in regulation of mammary development (Pausch *et al.*, 2016). Moreover, Flury (2014) reported a genome-wide association study for 13 udder morphology traits using imputed high-density genotypes in Brown Swiss sires. Five significant signals that affect different morphological features of mammary gland were detected, as well as one region located on chromosome six which was associated with clinical mastitis and milking speed. However, no genetic characteristics were reported for buffalo mammary gland morphology.

In this study, we performed genome-wide association study with 90 K Affymetrix Axiom Buffalo SNP Array to identify candidate genes and QTL that affect different morphological features of buffalo mammary gland. Five traits were used as response variables in 176 genotyped buffaloes. Our results are potentially beneficial for understanding the genetic regulation of buffalo mammary gland morphology.

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Materials and methods

Animals and phenotypic records

The Ethical Animal Care and Use Committee of Federico II University of Naples (Italy) approved the experimental design and animal treatment.

A total of 176 Italian Mediterranean buffaloes born from 2005 to 2011 within at least one lactation were used for this study. The buffalo mammary gland morphology, including

anterior teat length (ATL), posterior teat length (PTL), distance between anterior teats (AAD), distance between anterior and posterior teat (APD) and distance between posterior teats (PPD), were measured after milking, and every index were measured twice at different times of 1 d.

Genotyping and quality control

The DNAs were genotyped using the 90 K Axiom Buffalo SNP Array at Delta Genomics (Edmonton AB, Canada). Subsequently, quality control was performed using PLINK software version 1.9. Single nucleotide polymorphisms (SNPs) were excluded from the data set if they exhibited: (1) animal call rate <97%; (2) SNP call rate <95%; (3) minor allele frequency <0.05; (4) Hardy–Weinberg equilibrium (HWE) test P -value < 10^{-6} ; and (5) chromosome and position were unknown.

Single trait genome wide association studies

Buffalo with both phenotypic data and genotype data were used to performed GWAS by using mixed model as following by both PLINK 1.07 and ASReml 2.0.

$$y_{ijklm} = \mu + \text{parity}_i + \text{LP}_j + \text{age}_k + \text{SNP}_l \text{animal}_m + e_{ijklm}$$

where y is the vector of phenotypes; μ is overall mean; parity_i is fixed effect of the i th parity; LP_j is the fixed effect of the j th lactation period (early, middle and late); age_k is the k th covariate of age at calving; SNP_l is fixed effect of the l th genotype of the SNP; animal_m is m th random animal effect and e is the random residual error. Fixed effects were only fitted if they made a significant contribution to the overall variance component ($P < 0.05$). False discovery rate (FDR) was used to adjust P value for all available SNPs, and the significant threshold of SNP was proposed as $\text{FDR} < 0.1$. The suggestive threshold was proposed as $P < 10^{-4}$.

Haplotype analyses

The linkage disequilibrium (LD) and haplotype frequencies were obtained by Haploview 4.2 software. Haplotype blocks were constructed by using PHASE software, and the association between each haplotype block and mammary gland morphology was estimated in R. We used suggestive P value ($P < 0.05$) as threshold for considering candidate haplotype blocks affecting buffalo mammary gland morphology.

Results

Genome-wide association studies

Descriptive statistical results of the five indicators were presented in Supplementary File Table S1. After filtering low quality data by quality control, a total of 63 605 available SNPs and 176 buffaloes remained for subsequent GWAS. With single marker and single trait association analysis between buffalo mammary gland morphology and SNPs for each trait (Supplementary File Fig. S1), a total of 79 suggestive SNPs ($P < 10^{-4}$) associated with at least one of five mammary gland morphology were identified (Supplementary File Table S2). After FDR adjustment, three SNPs (AX-85117983, AX-85094752, and AX-85117518) were significantly associated with buffalo ATL, PTL and APD, respectively. According to the

map information, the three mutations were located on chromosome 1, chromosome 10, and chromosome 20, respectively.

Candidate genomic regions

To further identify QTL associated with buffalo udder traits, linkage disequilibrium (LD) analyses were performed to determine the relationship of SNPs within 0.5Mb window around three significant SNPs. A total of three haplotype blocks were recognized (Fig. 1). For each block, the association with buffalo mammary gland morphology was evaluated (Table 1). The block_2 was found to have an effect on buffalo PTL ($P = 0.041$), and block_3 was showed to be associated with buffalo ATL ($P = 0.03$) as well as PPD ($P = 0.014$). However, the block_1 were showed to be no significant effect on all buffalo mammary gland morphology in the present study.

Candidate genes within identified genomic region

A total of 6 genes were discovered within the identified genomic region. Among the genes, *GJD2*, *ACTC1*, *AQR*, and *ZNF770* located in the block_2 region, *IPO11* and *DIMT1* in the block_3 region. The description of these genes is presented in Supplementary File Table S3.

Discussion

In the present study, two significant blocks located on the 10th chromosomes and 20th chromosome respectively showed effects on buffalo udder traits. Interestingly, the areas where the two blocks were located were reported to be within or near the QTLs of mastitis (Talstein *et al.*, 2010) and production life (Chamberlain *et al.*, 2012). Furthermore, the block on chromosome 20 detected in this study is in a genomic region that controls milk yield traits in cattle (Arranz *et al.*, 2015). However, we did not detect any SNP associated with buffalo udder traits on chromosome 5, which is a chromosome that is recognized to include many QTLs related to udder traits in dairy cattle and beef breeds.

Endocrine factors play a crucial role in the regulation of mammary growth (Rowson *et al.*, 2012). In the present study, a total of 6 genes were located on the significant region, and one endocrine-related gene was included. *GJD2* (*Gap Junction Protein Delta 2*), located on chromosomes 10 from 30.255 to 30.257 Mb, encodes a member of the connexin protein family, which may be involved in the regulation of insulin secretion (Valentina *et al.*, 2016). Insulin was considered to play a role in mammogenesis and lactation, since the concentration of insulin is related to the expression of leptin, which was detected in adipocytes, alveolar epithelial, and mammary epithelial cells (Çatlı *et al.*, 2014; Palin *et al.*, 2017). Therefore, we speculate that *GJD2* gene may affect mammogenesis in buffalo by regulating expression of insulin and leptin. Having said this, it should be pointed out that the major mammogenic endocrine signals comprise steroid hormones and polypeptide hormones of pituitary and placental origin.

ACTC1 (*Cardiac alpha-actin*) located on the block_2 region was identified to be associated with buffalo mammary gland morphology. *ACTC1* belongs to the actin family and plays a crucial role in the muscle formation of filaments. The polymorphisms of *ACTC1* were associated with hypertrophic cardiomyopathy (Despond and Dawson, 2018). Two studies of

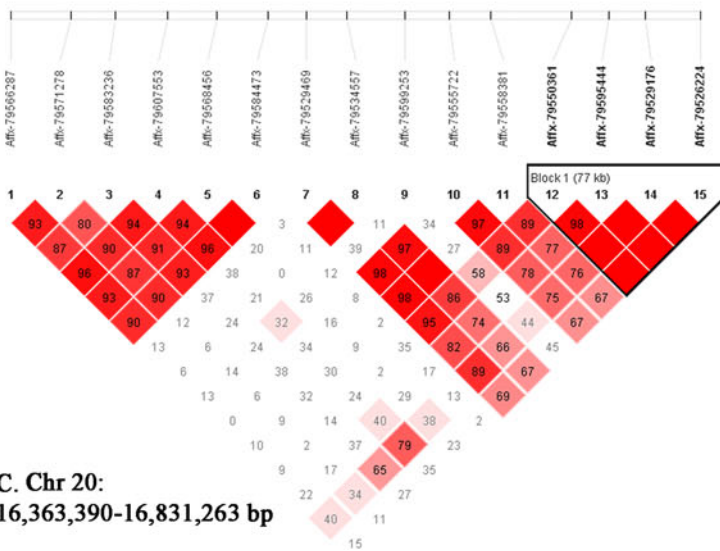
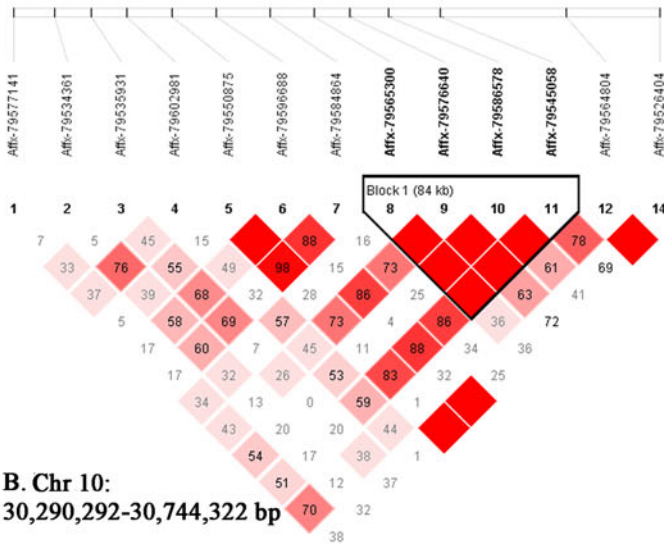
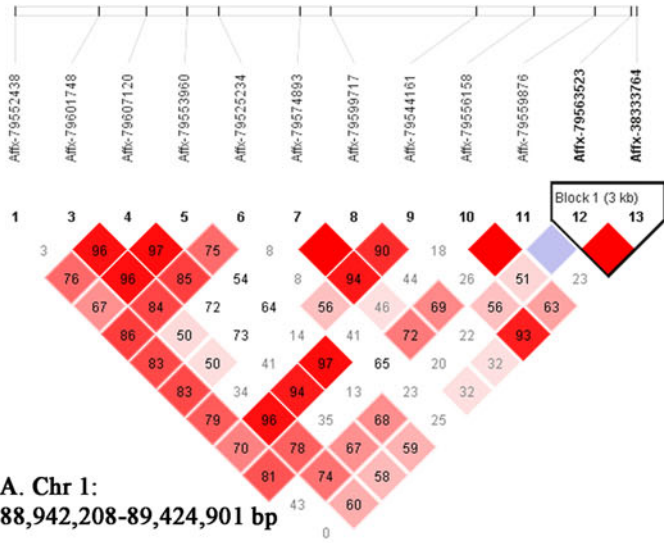


Fig. 1. Haplotype blocks pattern for the significant SNPs based on the linkage disequilibrium within detected regions (0.5 Mb). Linkage disequilibrium block is presented in triangle box based on LD (squared correlation coefficient, r^2).

selection signatures in dairy and beef cattle revealed many interesting candidate genes affecting milk production, body size and muscle formation, and both of them included *ACTC1* (Qanbari

et al., 2011; Zhao *et al.*, 2015). Moreover, *ACTC1* expressed in the retina and implicated as a candidate gene that influence the development of myopia (Solouki *et al.*, 2010). Interestingly, a

Table 1. The association between haplotypes and buffalo mammary gland morphology

Block	Haplotype	Frequency (%)	Traits				
			ATL	AAD	PTL	PPD	APD
Block_1	GC	70.2	0.968	0.328	0.783	0.391	0.438
	GT	5.5					
	TT	24.3					
Block_2	GGCT	34.9	0.408	0.217	0.041*	0.794	0.504
	AATC	34.4					
	AGTT	23.3					
	AGCT	5.7					
Block_3	TCCT	56.7	0.03*	0.677	0.721	0.014*	0.4
	CGCC	27.6					
	TCCC	8.1					

ATL, anterior teat length; PTL, posterior teat length; AAD, distance between anterior teats; APD, distance between anterior and posterior teat; PPD, distance between posterior teats.
* $P < 0.05$: significant association after Bonferroni multiple test.

gene associated with progressive vision loss was reported to be associated with udder structure in cattle breeds (Michot *et al.*, 2016), although the biological relevance of this observation is not clear.

The association between udder traits and milk production performance has been broadly corroborated, and many GWAS reported candidate genes may influence mammary development (Flury, 2014; Pausch *et al.*, 2016). However, most of these genes have a limited role in milk productive traits through effects on mammary growth, and many genes associated with milk productive traits were not found to be associated with udder traits, such as *DGAT1* (Li *et al.*, 2018), *PRL* (Li *et al.*, 2017), and *ABCG2* (Cohen-Zinder *et al.*, 2005). In the present study, none of candidate genes except *ACTC1* have obvious effects on milk production.

In conclusion, this study revealed candidate genes and potential genomic regions for mammary gland morphology in buffalo. To our knowledge, this is the first report about genetic basis of buffalo mammary gland morphology by genome-wide association study. These findings will provide a better understanding of the genetic regulation of mammary growth. However, development and lactation of mammary gland are complex biological process, and more data are needed to answer the question whether genetic improvement in mammary gland morphology will be beneficial for the productive performance.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0022029919000967>.

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References

- Arranz JJ, Coppeters W, Berzi P, Cambisano N, Grisart B, Karim L, Marcq F, Moreau L, Mezer C and Riquet J (2015) A QTL affecting milk yield and composition maps to bovine chromosome 20: a confirmation. *Animal Genetics* **29**, 107–115.
- Çatlı G, Olgaç Dündar N and Dündar BN (2014) Adipokines in breast milk: an update. *Journal of Clinical Research and Pediatric Endocrinology* **6**, 192.
- Chamberlain AJ, Hayes BJ, Savin K, Bolormaa S, McPartlan HC, Bowman PJ, Van der Jagt C, MacEachern S and Goddard ME (2012) Validation of single nucleotide polymorphisms associated with milk production traits in dairy cattle. *Journal of Dairy Science* **95**, 864–875.
- Cohen-Zinder M, Seroussi E, Larkin DM, Looor JJ, Everts-van A, Lee JH, Drackley JK, Band MR, Hernandez AG, Shani M, Lewin HA, Weller JI and Ron M (2005) Identification of a missense mutation in the bovine *ABCG2* gene with a major effect on the QTL on chromosome 6 affecting milk yield and composition in Holstein cattle. *Genome Research* **15**, 936–944.
- Davis SR (2017) TRIENNIAL LACTATION SYMPOSIUM/BOLFA: mammary growth during pregnancy and lactation and its relationship with milk yield. *Journal of Animal Science* **95**, 5675–5688.
- Despond EA and Dawson JF (2018) Classifying cardiac actin mutations associated with hypertrophic cardiomyopathy. *Frontiers in Physiology* **9**, 405.
- Flury C (2014) Genome-wide association study for 13 udder traits from linear type classification in cattle. 10th World Congress on Genetics Applied to Livestock Production.
- Li J, Liang AX, Li ZP, Du C, Hua GH, Salzano A, Campanile G, Gasparrini B and Yang LG (2017) An association analysis between PRL genotype and milk production traits in Italian Mediterranean river buffalo. *Journal of Dairy Research* **84**, 430–433.
- Li J, Liu S, Li Z, Zhang S, Hua G, Salzano A, Campanile G, Gasparrini B, Liang A and Yang L (2018) *DGAT1* polymorphism in riverine buffalo, swamp buffalo and crossbred buffalo. *Journal of Dairy Research* **85**, 412–415.
- Michot P, Cahory S, Marete A, Grohs C, Dagios D, Donzel E, Aboukadiri A, Deloche MC, Allais-Bonnet A, Chambrial M, Barbey S, Genestout L, Boussaha M, Danchin-Burge C, Fritz S, Boichard D and Capitan A (2016) A reverse genetic approach identifies an ancestral frameshift mutation in RPI causing recessive progressive retinal degeneration in European cattle breeds. *Genetics Selection Evolution* **48**, 56.
- Palin MF, Farmer C and Duarte CRA (2017) TRIENNIAL LACTATION SYMPOSIUM/BOLFA: adipokines affect mammary growth and function in farm animals. *Journal of Animal Science* **95**, 5689–5700.
- Pausch H, Emmerling R, Schwarzenbacher H and Fries R (2016) A multi-trait meta-analysis with imputed sequence variants reveals twelve QTL for mammary gland morphology in Fleckvieh cattle. *Genetics Selection Evolution* **48**, 14.
- Qanbari S, Gianola D, Hayes B, Schenkel F, Miller S, Moore S, Thaller G and Simianer H (2011) Application of site and haplotype-frequency based approaches for detecting selection signatures in cattle. *BMC Genomics* **12**, 318.
- Rowson AR, Daniels KM, Ellis SE and Hovey RC (2012) Growth and development of the mammary glands of livestock: a veritable barnyard of opportunities. *Seminars in Cellular and Developmental Biology* **23**, 557–566.

- Solouki AM, Verhoeven VJ, van Duijn CM, Verkerk AJ, Ikram MK, Hysi PG, Despriet DD, van Koolwijk LM, Ho L and Ramdas WD** (2010) A genome-wide association study identifies a susceptibility locus for refractive errors and myopia at 15q14. *Nature Genetics* **42**, 897–901.
- Talstein R, Fontanesi L, Dolezal M, Scotti E, Bagnato A, Russo V, Canavesi F, Friedmann A, Soller M and Lipkin E** (2010) A genome scan for quantitative trait loci affecting milk somatic cell score in Israeli and Italian Holstein cows by means of selective DNA pooling with single- and multiple-marker mapping. *Journal of Dairy Science* **93**, 4913–4927.
- Valentina C, Celine P, Pierri CL, Samuel D, Jacques-Antoine H, João F, Valeriya L, Leif G, Rico R and Fabrizio T** (2016) A variant of GJD2, encoding for connexin 36, alters the function of insulin producing β -cells. *PLoS ONE* **11**, e0150880.
- Zhao F, McParland S, Kearney F, Du L and Berry DP** (2015) Detection of selection signatures in dairy and beef cattle using high-density genomic information. *Genetics Selection Evolution* **47**, 49.