Indian Journal of Animal Sciences **96** (5): 398–403, May 2026/Article
https://doi.org/10.56093/ijans.v96i5.178691

Transcriptome-wide association study identifies candidate genomic regions for lactation persistency in Murrah buffaloes

PANKAJ CHAYAL[1], VIKAS VOHRA[1]✉, SUPRIYA CHHOTARAY[2], AKSHATA PATIL[1], HEMLATA VALMIKI[1] and DAMINI SHARMA[2]

ICAR-National Dairy Research Institute, Karnal 132 001 Haryana India

Received: 05 May 2026; Accepted: 30 June 2026

ABSTRACT

Lactation persistency is an important economic trait of dairy animals. Understanding the genetic architecture underlying lactation persistency remains a challenge, as conventional genome-wide association studies (GWAS) often lack functional interpretability. The present study aimed to identify genes associated with lactation persistency in Murrah buffaloes using a transcriptome-wide association study (TWAS) approach. Test-day milk yield records from 120 Murrah buffaloes up to 245 days post-calving were used to model lactation curves using Wood's incomplete gamma function. The post-peak decline rate was considered as a measure of lactation persistency to perform TWAS. TWAS was conducted using an already predicted gene expression panel comprising 26,956 genes. No gene-level association surpassed the Bonferroni-corrected genome-wide significance threshold or the suggestive significance threshold. Although the limited sample size reduced power for detection and no statistically significant association was observed, functional annotation of the top-ranked genes based on their lowest p-values suggested their involvement in biological processes related to mammary epithelial cell survival, proliferation, and differentiation, which are critical for maintaining milk production after peak lactation. The present exploratory analysis highlighted potential genes and pathways that may contribute to regulation of lactation persistency in buffaloes. Further validations could substantiate these genes as candidates for genetic selection in Murrah buffaloes.

Keywords: Lactation persistency, Murrah, Post-peak decline rate, TWAS

Lactation is a multifaceted process that includes milk synthesis, secretion, and various stages of mammary gland development (Mukherjee *et al.* 2023). To maintain economic performance of a dairy farm, lactation persistency is an important trait. Lactation persistency can be defined as the ability of an animal to maintain milk production at a high level after reaching the peak milk production (Sehested *et al.* 2019). Heritability estimates for various definitions of persistency range from 0.09 to 0.30 (Cole and Null 2009, Muir *et al.* 2004). The shape of the lactation curve provides important insights into the productive potential of dairy animals. Among its features, the decline rate following peak yield serves as a critical indicator of lactation persistency. High lactation persistency is associated with a slow rate of production decline, whereas low lactation persistency is associated with a rapid rate of production decline (Cole and Null 2009). Animals that decline more slowly maintain milk production for a longer period, thereby ensuring greater cumulative yield and improved production efficiency. Hence, the decline rate of the lactation curve is widely recognized as a reliable measure of lactation persistency.

In previous years, considerable efforts have been made to dissect the genetic basis of lactation persistency using genome-wide association studies (Do *et al.* 2017, Nayeri *et al.* 2017, Pedrosa *et al.* 2021, Vohra *et al.* 2021). Despite this success, genome-wide association studies (GWAS) face significant challenges in delivering clear biological interpretation. A major limitation is that most GWAS-identified variants reside in non-coding regions of the genome, making it difficult to infer their direct functional roles (Abdalla *et al.* 2022, Visscher *et al.* 2017). Consequently, the candidate genes and underlying molecular mechanisms involved are often unclear, limiting the interpretability of GWAS findings (Cano-Gamez and Trynka 2020). Transcriptome-wide association studies (TWAS) provide an alternative framework for directly assessing the association between genes and phenotypic variation, leveraging fine-mapping of expression quantitative trait loci (eQTLs) and the identification of candidate genes with higher power than conventional genome-wide association studies (Chhotaray *et al.* 2023). While GWAS relies on genotypes as causal variables, TWAS uses predicted gene expression levels as predictors of phenotypic variation (Cao *et al.* 2021). Hence, the present study was conducted to identify putative genes

Present address: [1]Animal Genetics and Breeding, ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India.[2] Animal Genetics and Breeding, ICAR- Central Institute for Research on Buffaloes, Hisar-125 001, Haryana, India. ✉Corresponding author email: vohravikas@gmail.com

associated with lactation persistency in Murrah buffaloes.

MATERIALS AND METHODS

Preparation of the dataset: Monthly test-day milk records up to 245 days post-calving for 120 Murrah buffaloes belonging to first lactation were collected from Animal Genetics and Breeding Division, National Dairy Research Institute, Karnal, India (29.68°N and 76.99°E).

Lactation curves were modelled using incomplete gamma function given by Wood (1969) and described by Macciota *et al.* (2005) as:

$$Y_t = at^b e^{ct}$$

where represented milk yield at time (days in milk), denoted the initial milk yield, describes the ascending phase of the lactation curve, and represented the rate of decline after peak yield. The parameters of the lactation curve were estimated for individual animals, and lactation persistency was assessed based on the declining rate of the curve after peak yield. The curve parameters were estimated using R package "minpack.lm" v1.2-4. All 120 animals were retained for downstream TWAS analysis.

Transcriptome-wide Association Study: A predicted gene expression panel generated in the Buffalo Breeding Lab, Animal Genetics and Breeding Division, National Dairy Research Institute, Karnal, was used for association analysis. The panel contained predicted expression values for 26,956 genes and was developed through the integration of genomic and transcriptomic information using the Bayesian Dirichlet Process Regression (DPR) approach implemented in TIGAR (Chhotaray *et al.* 2023). The DPR models achieved an average training accuracy (R^2) of 0.49 and an average cross-validation accuracy (R^2) of 0.48, indicating good predictive performance. The animals used for development of the gene expression prediction panel were independent of the 120 Murrah buffaloes included in the present study, and therefore no overlap existed between the model-training and association-testing datasets. The genotype quality control procedures, SNP filtering criteria, imputation strategy, and expression prediction model development are described in detail by Chhotaray *et al.* (2023), from whom the prediction panel used in the present study was obtained. The transcriptomic data was derived from mammary epithelial cells isolated from milk samples. The present study utilized these precomputed genetically predicted gene expression without further modification The association between predicted gene expression and the quantitative trait was evaluated using the linear regression framework implemented in the TIGAR v1.0 software (Nagpal *et al.* 2019). The following statistical model was used (Nagpal *et al.* 2019):

$$f(E[Y|X,C]) = \eta C + \beta GReX$$

where, f(.) was a pre-specified link function, which is set as identity function for the quantitative phenotype, [Y|X,C]: Phenotype given genotype matrix X_{test} and covariate matrix C, η represented the vector of covariate effects corresponding to the covariate matrix C, *GreX* denoted the genetically regulated predicted gene expression, β represented the association effect of genetically regulated predicted gene expression, H_0: β = 0. Age at first calving (AFC) and birth weight were included as covariates to account for potential non-genetic sources of variation influencing lactation persistency. AFC is a well-recognized factor affecting first-lactation performance, whereas birth weight reflects early-life growth and developmental status that may influence subsequent production traits. The effect of both the non-genetic factors considered as covariates in the present study, were significant on the lactation persistency ($p < 0.05$), supporting their inclusion in the association model.

A genome-wide significance threshold of 1.8547×10^{-6} was established using Bonferroni correction (0.05/26,956 genes). In addition, false discovery rate (FDR)–adjusted p-values were estimated using the Benjamini–Hochberg procedure to provide a less stringent assessment of statistical significance. Gene coordinates and genomic annotations were based on the *Bubalus bubalis* reference genome assembly GCF_019923935.1 (NDDB_SH_1), consistent with the prediction panel developed by Chhotaray *et al.* (2023). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotations were obtained using the Database for Annotation, Visualization and Integrated Discovery (DAVID) platform to explore the functional roles of the top-ranked genes (Huang *et al.* 2009).

RESULTS AND DISCUSSION

Lactation persistency, estimated as the declining slope parameter (*c*) as described by Macciotta *et al.* (2005), had a Mean ± SD of –0.00559 ± 0.00370 (N = 120), with a minimum and maximum values of –0.01663 and 0.00132, respectively and a coefficient of variation of 66.19%. Prior to conducting the TWAS analysis, the distribution of the Wood's c parameter was assessed. The c parameter exhibited a unimodal distribution, with most observations clustered in the central range, although a moderate deviation was observed in the lower tail (Supplementary file). Given that this parameter reflects a continuous aspect of the lactation curve and no pronounced outliers or distinct groupings were identified, the original c values were maintained for subsequent association analysis.

TWAS results are visualised as a Manhattan plot (Fig. 1) and Q-Q plot (Fig. 2) created in R using package "qqman" (Turner 2014). None of the genes surpassed the Bonferroni-corrected genome-wide significance threshold or the suggestive significance threshold. None of the tested genes surpassed the Bonferroni-corrected or suggestive significance thresholds. Therefore, the reported genes represented exploratory candidate genes and should be interpreted as hypothesis-generating associations. The top 20 genes having the lowest *P*-value were identified and are given in Table 1. Functional annotation of the top-ranked genes revealed their involvement in biological processes

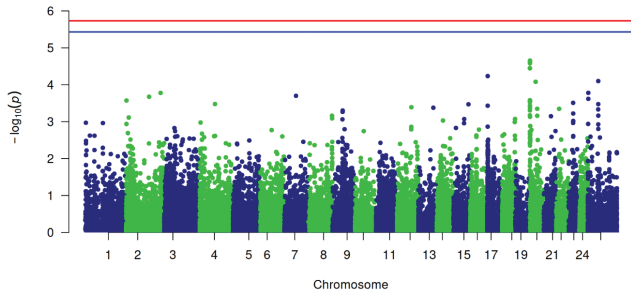

Fig. 1. Manhattan plot showing the results for association with lactation persistency. The red line indicates genome-wide *p*-value threshold (expressed as $-\log_{10}P$) corresponding to Bonferroni corrected *p*-values (1.8547×10^{-6}). The blue line indicates the genome-wide suggestive threshold corresponding to Bonferroni corrected *p*-values (3.71×10^{-6}).

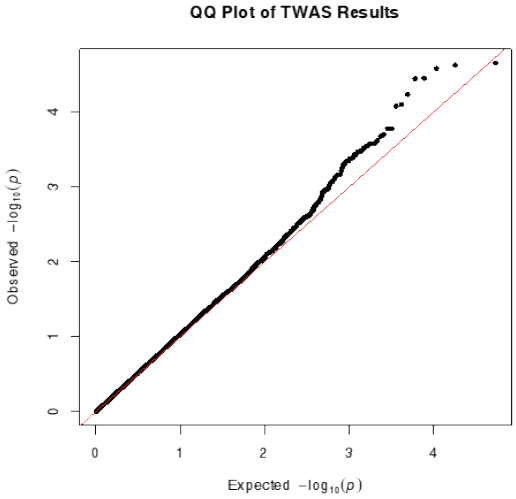

Fig. 2. Q-Q plot showing the results for association with lactation persistency where observed $-\log_{10}P$ values are plotted against the expected $-\log_{10}P$.

such as apoptosis, cell proliferation, transcriptional regulation, and signal transduction. Detailed GO terms and KEGG pathway annotations are provided in Supplementary File S1.

Lactation persistency is defined by the rate of decline in milk yield after peak lactation and is primarily governed by changes in number of mammary epithelial cell (MEC), only cell type that produces milk during lactation. The prevailing evidence indicated that declining milk yield was largely attributable to an imbalance between cell proliferation and programmed cell death (apoptosis) rather than reduced activity of individual cells (Capuco *et al.* 2003, Lund *et al.* 1996). After peak lactation, the progressive loss of secretory MECs through apoptosis leads to mammary gland regression and reduced lactation persistency (Stefanon *et al.* 2002). In the present study, several top ranked sub-threshold signals were observed on the chromosome 20, with a single peak with trailing SNPs. This clustering might be indicative of a regional genomic signal for lactation persistency. Previously, Vohra *et al.* (2021) have reported SNPs on the chromosome 20 to be

Table 1. Top 20 genes identified through TWAS having lowest p-value suggesting probable association with lactation persistency

CHR	Gene start	Gene end	Gene name	Description	P value	FDR
20	1325899	1330215	SIVA1	SIVA1 apoptosis inducing factor	2.21×10^{-5}	0.193
20	778736	782922	TMEM121	transmembrane protein 121	2.37×10^{-5}	0.193
20	1141190	1149722	CDCA4	cell division cycle associated 4	2.62×10^{-5}	0.193
20	578871	588448	LOC102400566	immunoglobulin heavy constant delta	3.55×10^{-5}	0.193
20	985911	988453	BTBD6	BTB domain containing 6	3.58×10^{-5}	0.193
17	1859100	1865284	LOC123465052	zinc finger protein 280A-like	5.82×10^{-5}	0.261
25	51180588	51185332	LOC123331963	uncharacterized	7.95×10^{-5}	0.279
20	28901010	28914353	COCH	cochlin	8.3×10^{-5}	0.279
25	2151908	2162437	LOC123331826	uncharacterized	1.65×10^{-4}	0.414
2	170494822	170576142	SP140	SP140 nuclear body protein	1.66×10^{-4}	0.414
7	56234328	56354047	LOC123334431	uncharacterized	1.99×10^{-4}	0.414
2	113848128	113848548	LOC112578469	translation machinery-associated protein 7-like	2.11×10^{-4}	0.414
25	2147432	2200136	LOC123331825	uncharacterized	2.41×10^{-4}	0.414
20	813473	818553	LOC102409763	cysteine-rich protein 2	2.63×10^{-4}	0.414
20	1024742	1031916	NUDT14	nudix hydrolase 14	2.64×10^{-4}	0.414
2	2589451	2606381	LOC123328728	uncharacterized	2.67×10^{-4}	0.414
20	1036947	1058561	JAG2	jagged canonical Notch ligand 2	2.82×10^{-4}	0.414
20	876637	940562	PACS2	phosphofurin acidic cluster sorting protein 2	2.98×10^{-4}	0.414
23	21641289	21647035	NDUFB8	NADH:ubiquinone oxidoreductase subunit B8	3.09×10^{-4}	0.414
4	75216121	75260970	RAP1B	RAP1B, member of RAS oncogene family	3.33×10^{-4}	0.414

associated with milk yield. Chhotaray *et al.* (2023) also reported GWAS signals for milk yield on chromosome 20. They reported TWAS signals at ~11 Mbp on chromosome 20 for milk yield. Although the positional overlap between the reported studies and the present findings could not be established, the role of chromosome 20 in regulation of production traits in buffaloes is evident.

In present study, the SIVA1 gene was identified as being involved in extrinsic apoptotic signaling pathway. This pathway has been demonstrated to be involved in mammary gland development and lactation persistency (Do *et al.* 2017, Green and Streuli 2004, Lund *et al.* 2000, Watson 2006). In addition, CDCA4, a gene known to regulate cell cycle progression and apoptosis, has been reported to inhibit apoptosis in Wilms' tumour cells (Li *et al.* 2021) and to significantly influence both cell proliferation and apoptotic processes in human triple-negative breast cancer (Zeng *et al.* 2019). CDCA4 has been identified in previous studies of non-ruminant species as a gene associated with cell cycle regulation and cellular proliferation. These biological processes are fundamental to the turnover of mammary epithelial cells and the maintenance of lactation. However, there is currently no direct evidence linking CDCA4 to lactation persistency in buffalo populations. Similarly, TMEM121 has been mainly characterized in cancer and cardiac biology, where it functions as an inhibitor of tumour metastasis (Yang *et al.* 2022) and pathological cardiac hypertrophy (Xu *et al.* 2016), its role in regulating cell signaling and growth suggests broader relevance to tissue homeostasis. Although TMEM121 has been identified in studies involving non-ruminant species, particularly regarding epithelial cell behavior and tissue remodeling, its specific function in bovine or buffalo mammary gland biology remains to be elucidated. At present, there is no direct evidence establishing its role in these species. However, lactation persistency depends on mammary epithelial cell survival, proliferation, and apoptotic balance during post-peak lactation. Therefore, the identification of TMEM121 in the present study suggests that it may contribute to mammary epithelial cell stability and longevity, potentially influencing sustained milk production.

JAG2 gene has been reported to be associated with Notch signaling pathway. This pathway has been previously reported to be involved in milk lactose metabolism and play a crucial role in mammary gland development and lactation (Politi *et al.* 2004), as it controls mammary epithelial cell fate (Yalcin-Ozuysal *et al.* 2010). Suppression of apoptosis of human mammary epithelial cells by this pathway has already been reported (Meurette *et al.* 2009). NOTCH-1 and -3 and their ligands, JAG-1 and -2, were most prominently expressed in the luminal epithelium of the normal human breast. This gene is involved in the establishment or maintenance of epithelial cell apical/basal polarity. Apical-basal polarity is essential for epithelial cell form and function, as it determines the localization of the adhesion molecules that hold the cells together laterally and the occluding junctions that act as barriers to paracellular diffusion. Polarity must also target the secretion of specific cargoes to the apical, lateral or basal membranes and organize the cytoskeleton and internal architecture of the cell (Buckley and Johnston 2022). Furthermore, *JAG2* has been reported to be associated with fat yield in Thai multibreed dairy cattle (Laodim *et al.* 2023). Notably, *COCH* has been previously reported in the BuffaloQTLdb and has been associated with milk yield and protein percentage in buffalo populations (Deng *et al.* 2019, Deng *et al.* 2024). The overlap between the present TWAS findings and previously reported production-trait associations provided additional support for the potential biological relevance of this gene in lactation-related processes.

Ras-associated proteins (Rap) define a family of highly homologous small guanosine triphosphate (GTP)–binding proteins belonging to the Ras superfamily. *Rap1B* gene regulates dynamic changes in cell adhesion (Wilson *et al.* 2016) and its involvement in MAPK pathway, Rap1 signaling pathway and Ras signaling pathway suggested its role in regulation of milk production as, these are the core pathways of mammary cell proliferation and differentiation which is essential for lactogenesis and mammary gland function (Farhadian *et al.* 2021, Fata *et al.* 2007, Hu *et al.* 2024, Itoh *et al.* 2007, Mu *et al.* 2022).

Overall, the integration of apoptosis-related genes with key signaling pathways governing mammary epithelial cell fate, polarity, adhesion, and proliferation highlighted the coordinated molecular regulation underlying lactation persistency. These findings provided functional context for the identified candidate genes and supported their biological relevance to sustained milk production. Despite providing novel insights into the genetic basis of lactation persistency in Murrah buffaloes, this study has certain limitations. No statistically significant gene-level associations were detected and all candidate genes reported here were sub-threshold and require further validation. The relatively modest sample size may have limited the statistical power to detect genes with small effects. However, similar sample sizes have been reported in buffalo genomic studies due to limited availability of well-characterized populations with integrated genotype and phenotype data (Vohra *et al.* 2021, Chhotaray *et al.* 2023). In addition, gene-wise prediction accuracy statistics, along with gene wise heritability would have offered a better insight into the efficiency of association with predicted gene expression panel. Functional validation through gene expression profiling and experimental studies would further strengthen the biological interpretation and facilitate the application of these findings in genetic improvement programs.

This study applied a transcriptome-wide association approach to investigate lactation persistency in Murrah buffaloes and identified several top-ranked putative genes that may be involved in biological processes relevant to mammary gland function. However, no statistically significant gene-level associations were detected after

multiple-testing correction. Therefore, all reported genes in the present study should be considered exploratory and requires independent replication and functional validation in buffaloes.

ACKNOWLEDGEMENT

The research was supported by the Indian Council of Agricultural Research, Department of Agricultural Research and Education, Govt. of India. The authors thank the Director(s) of ICAR-NDRI, Karnal, and ICAR-CIRB, Hisar for their guidance and support. The authors also gratefully acknowledge the Network Project on Buffaloes and the CABin Project, ICAR–CIRB, for providing essential facilitation for successful completion of this study.

Ethical approval: Previously generated and reported dataset from our lab was used to conduct the present study, hence, no additional ethical approval was required.

REFERENCES

Abdalla M and Abdalla M. 2022. A general framework for predicting the transcriptomic consequences of non-coding variation and small molecules. *PLOS Computational Biology* **18**(4): p.e1010028.

Buckley CE and St Johnston D. 2022. Apical–basal polarity and the control of epithelial form and function. *Nature Reviews Molecular Cell Biology* **23**(8): 559–577.

Cano-Gamez E and Trynka G. 2020. From GWAS to function: using functional genomics to identify the mechanisms underlying complex diseases. *Frontiers in Genetics* **11**: 424.

Cao C, Ding B, Li Q, Kwok D, Wu J and Long Q. 2021. Power analysis of transcriptome-wide association study: Implications for practical protocol choice. *PLOS Genetics* **17**(2): p.e1009405.

Capuco AV, Ellis SE, Hale SA, Long E, Erdman RA, Zhao X and Paape MJ. 2003. Lactation persistency: insights from mammary cell proliferation studies. *Journal of Animal Science* **81**(15): 18–31.

Chhotaray S, Vohra V, Uttam V, Santhosh A, Saxena P, Gahlyan RK and Gowane G. 2023. TWAS revealed significant causal loci for milk production and its composition in Murrah buffaloes. *Scientific Reports* **13**(1): 22401.

Cole JB, Null DJ and VanRaden PM. 2009. Best prediction of yields for long lactations. *Journal of Dairy Science* **92**(4): 1796–1810.

Deng TX, Ma XY, Duan A, Lu XR and Abdel-Shafy H. 2024. Genome-wide copy number variant analysis reveals candidate genes associated with milk production traits in water buffalo (Bubalus bubalis). *Journal of Dairy Science* **107**(9): 7022–7037.

Deng T, Liang A, Liang S, Ma X, Lu X, Duan A, Pang C, Hua G, Liu S, Campanile G and Salzano A. 2019. Integrative analysis of transcriptome and GWAS data to identify the hub genes associated with milk yield trait in buffalo. *Frontiers in Genetics* **10**: 36.

Do DN, Bissonnette N, Lacasse P, Miglior F, Sargolzaei M, Zhao X and Ibeagha-Awemu EM. 2017. Genome-wide association analysis and pathways enrichment for lactation persistency in Canadian Holstein cattle. *Journal of Dairy Science* **100**(3): 1955–1970.

Farhadian M, Rafat SA, Panahi B and Mayack C. 2021. Weighted gene co-expression network analysis identifies modules and functionally enriched pathways in the lactation process. *Scientific Reports* **11**(1): 2367.

Fata JE, Mori H, Ewald AJ, Zhang H, Yao E, Werb Z and Bissell MJ. 2007. The MAPKERK-1, 2 pathway integrates distinct and antagonistic signals from TGFα and FGF7 in morphogenesis of mouse mammary epithelium. *Developmental Biology* **306**(1): 193–207.

Hu Z, Lu Y, Cai J, Liu J and Wang D. 2024. Cross-oxygen gradients transcriptomic comparison revealed the central role of MAPK and hippo in hypoxia-mediated mammary proliferation inhibition. *Antioxidants* **13**(3): 288.

Huang DW, Sherman BT and Lempicki RA. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols* **4**(1): 44–57.

Itoh M, Nelson CM, Myers CA and Bissell MJ. 2007. Rap1 integrates tissue polarity, lumen formation, and tumorigenic potential in human breast epithelial cells. *Cancer Research* **67**(10): 4759–4766.

Laodim T, Koonawootrittriron S, Elzo MA, Suwanasopee T, Jattawa D and Sarakul M. 2023. Genetic factors influencing milk and fat yields in tropically adapted dairy cattle: insights from quantitative trait loci analysis and gene associations. *Animal Bioscience* **37**(4): 576.

Li S, Qin C, Chen Y, Wei D, Tan Z and Meng J. 2021. Implications of cell division cycle associated 4 on the Wilm's tumor cells viability via AKT/mTOR signaling pathway. *Renal Failure* **43**(1): 1470–1478.

Lund LR, Bjørn SF, Sternlicht MD, Nielsen BS, Solberg H, Usher PA, Østerby R, Christensen IJ, Stephens RW, Bugge TH and Danø K. 2000. Lactational competence and involution of the mouse mammary gland require plasminogen. *Development* **127**(20): 4481–4492.

Lund LR, Rømer J, Thomasset N, Solberg H, Pyke C, Bissell MJ, Danø K and Werb Z. 1996. Two distinct phases of apoptosis in mammary gland involution: proteinase-independent and-dependent pathways. *Development* **122**(1): 181–193.

Macciotta NPP, Vicario D and Cappio-Borlino A. 2005. Detection of different shapes of lactation curve for milk yield in dairy cattle by empirical mathematical models. *Journal of Dairy Science* **88**(3): 1178-1191.

Meurette O, Stylianou S, Rock R, Collu GM, Gilmore AP and Brennan K. 2009. Notch activation induces Akt signaling via an autocrine loop to prevent apoptosis in breast epithelial cells. *Cancer Research* **69**(12): 5015–5022.

Mu T, Hu H, Ma Y, Wen H, Yang C, Feng X, Wen W, Zhang J and Gu Y. 2022. Identifying key genes in milk fat metabolism by weighted gene co-expression network analysis. *Scientific Reports* **12**(1): 6836.

Muir BL, Fatehi J and Schaeffer LR. 2004. Genetic relationships between persistency and reproductive performance in first-lactation Canadian Holsteins. *Journal of Dairy Science* **87**(9): 3029–3037.

Das PK, Sejian V, Mukherjee J and Banerjee D. eds. 2023. *Textbook of veterinary physiology*: 513–568. Singapore: Springer.

Nagpal S, Meng X, Epstein MP, Tsoi LC, Patrick M, Gibson G, De Jager PL, Bennett DA, Wingo AP, Wingo TS and Yang J. 2019. TIGAR: an improved Bayesian tool for transcriptomic data imputation enhances gene mapping of complex traits. *The American Journal of Human Genetics* **105**(2): 258–266.

Nayeri S, Sargolzaei M, Abo-Ismail MK, Miller S, Schenkel F, Moore SS and Stothard P. 2017. Genome-wide association study for lactation persistency, female fertility, longevity, and

lifetime profit index traits in Holstein dairy cattle. *Journal of Dairy Science* **100**(2): 1246–1258.

Pedrosa VB, Schenkel FS, Chen SY, Oliveira HR, Casey TM, Melka MG and Brito LF. 2021. Genomewide association analyses of lactation persistency and milk production traits in Holstein cattle based on imputed whole-genome sequence data. *Genes* **12**(11): 1830.

Politi K, Feirt N and Kitajewski J. 2004. Notch in mammary gland development and breast cancer. In *Seminars in Cancer Biology* **14**(5): 341–347.

Sehested J, Gaillard C, Lehmann JO, Maciel GM, Vestergaard M, Weisbjerg MR, Mogensen L, Larsen LB, Poulsen NA and Kristensen T. 2019. Extended lactation in dairy cattle. *Animal* **13**(S1): 65–74.

Stefanon B, Colitti M, Gabai G, Knight CH and Wilde CJ. 2002. Mammary apoptosis and lactation persistency in dairy animals. *Journal of Dairy Research* **69**(1): 37–52.

Turner SD. 2014. qqman: an R package for visualizing GWAS results using QQ and manhattan plots. *Biorxiv*: 005165.

Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA and Yang J. 2017. 10 years of GWAS discovery: biology, function, and translation. *The American Journal of Human Genetics* **101**(1): 5–22.

Vohra V, Chhotaray S, Gowane G, Alex R, Mukherjee A, Verma A and Deb SM. 2021. Genome-wide association studies in Indian Buffalo revealed genomic regions for lactation and fertility. *Frontiers in Genetics* **12**: p.696109.

Wilson JM, Prokop JW, Lorimer E, Ntantie E and Williams CL. 2016. Differences in the phosphorylation-dependent regulation of prenylation of Rap1A and Rap1B. *Journal of Molecular Biology* **428**(24): 4929–4945.

Wood PDP. 1969. Factors affecting the shape of the lactation curve in cattle. *Animal Science* **11**(3): 307–316.

Xu W, Wang Y, Zhou J, Zhu X, Zhang S, Yuan W, Liu X, Shi Y, Cao L, Zeng Q and Jiang Z. 2016. Cardiac Specific Overexpression of hHole Attenuates Isoproterenol–Induced Hypertrophic Remodeling through Inhibition of Extracellular Signal-Regulated Kinases (ERKs) Signalling. *Current Molecular Medicine* **16**(5): 515–523.

Yalcin-Ozuysal Ö, Fiche M, Guitierrez M, Wagner KU, Raffoul W and Brisken C. 2010. Antagonistic roles of Notch and p63 in controlling mammary epithelial cell fates. *Cell Death and Differentiation* **17**(10): 1600–1612.

Yang B, Cai Y, Zhu P, Jiang Z, Ao J, Zhang Q, Yuan W, Peng Z, Chen J, Wen Y and Chen Y. 2022. Transmembrane protein 121 as a novel inhibitor of cervical cancer metastasis. *Experimental and Therapeutic Medicine* **24**(3): 572.

Zeng Y, Gao T, Huang W, Yang Y, Qiu R, Hou Y, Yu W, Leng S, Feng D, Liu W and Teng X. 2019. MicroRNA-455-3p mediates GATA3 tumor suppression in mammary epithelial cells by inhibiting TGF-β signaling. *Journal of Biological Chemistry* **294**(43): 15808–15825.