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Identification of a functional single nucleotide polymorphism in the *FASN* promoter associated with milk fat traits in dairy cattle

In the past decades, multiple genome-wide association studies have been performed to identify loci affecting milk production traits, including some using imputed whole-genome sequence data (i.e. Daetwyler et al., 2014; Fang & Pausch, 2019; Sanchez et al., 2017). Several genome-wide association studies have linked a polymorphism (rs136067046, BTA19: g.50775172 C>G on ARS-UCD1.2) located in the upstream region of *FASN* (encoding fatty acid synthase) to milk fat traits in dairy cattle (Cai et al., 2020; Sanchez et al., 2019; Tribout et al., 2020). For example, Cai and collaborators showed that rs136067046 is associated to milk fat traits in Nordic Holstein cows. *FASN* is an enzyme that plays a critical role in de novo fatty acid synthesis in milk. Interestingly, rs136067046 is located within an ATAC

peak (chr.19: 50 773 523–50 795 886) found in the mammary gland (Yuan et al., 2023). It is thus possible that rs136067046 is a functional variant that directly impacts these traits.

The ability of this SNP to alter transcription factor binding sites was then predicted with a custom script, as previously described (Ramírez-Ayala et al., 2021). This analysis suggests that rs136067046 modifies the binding sites of nine transcription factors (Table 1). Five of those transcription factors are expressed (transcript per million reads ≥ 0.5) in mammary gland tissue (Fang et al., 2020). Interestingly, three of those five transcription factors are from the Krüppel-like factor (KLF) family, a conserved class of transcription factors. It has been shown that KLF4 promotes milk fat synthesis in bovine mammary

TABLE 1 Prediction of the transcription factor binding sites altered by rs136067046.

SNP ID	Transcription factor	Database	Effect	Strand	DNA sequence	Matrix	Mammary gland expression ^a
rs136067046	Klf1	Jaspar	Gain	–	GGCCAGGCCCT	MA0493.1	0.106
rs136067046	KLF1_HUMAN	Hocomoco10	Gain	+	CAGGGCCTGGC	M01291	
rs136067046	Klf12	Jaspar	Gain	–	GGCCAGGCCCTGCAC	MA0742.1	0.487
rs136067046	KLF12_HUMAN	Hocomoco10	Gain	+	CAGGGCCTGGCC	M01286	
rs136067046	Klf4	Jaspar	Gain	+	AGGGCCTGGC	MA0039.2	5.786
rs136067046	KLF4_HUMAN	Hocomoco10	Gain	+	AGGGCCTGGC	M01293	
rs136067046	KLF5	Jaspar	Gain	–	GCCAGGCCCT	MA0599.1	9.837
rs136067046	KLF6_HUMAN	Hocomoco10	Loss	+	AGGGCCTG	M01294	4.508
rs136067046	KLF8_HUMAN	Hocomoco10	Gain	+	CAGGGCCTG	M01295	0.075
rs136067046	SP3	Jaspar	Gain	–	GCCAGGCCCTG	MA0746.1	11.641
rs136067046	SP8	Jaspar	Gain	–	GCCAGGCCCTGC	MA0747.1	0.001
rs136067046	ZFX_HUMAN	Hocomoco10	Loss	–	CTGGTGGCCAGGCCCTGCA	M01615	7.073
rs136067046	Zfx	Jaspar	Loss	+	CCGTGCAGGGCCTG	MA0146.2	

^aMean transcript per million reads (based on 661 mammary gland samples).

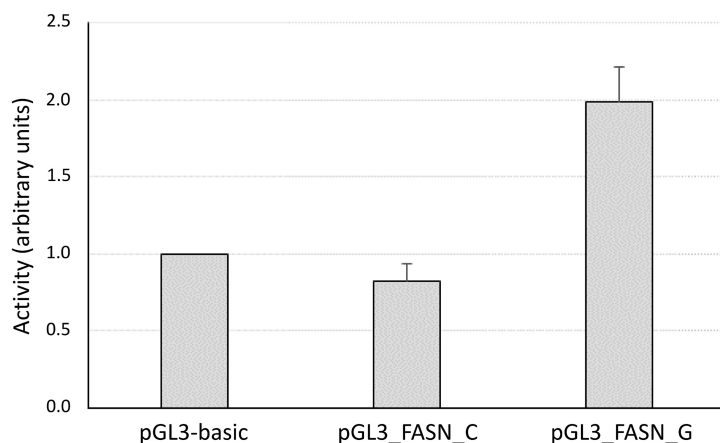


FIGURE 1 Results of luciferase reporter assays.

epithelial cells by targeting the *FASN* promoter region (Wu et al., 2024). The authors have shown, using yeast one-hybrid assay, that KLF4 interacts directly with a part of the *FASN* promoter region, encompassing the location of rs136067046. It has also been shown that KLF5 controls the expression of *FASN* through an interaction with SREBBP-1 (Lee et al., 2009). In addition, inactivation of *KLF6* in bovine mammary epithelial cells increases *FASN* expression (Iqbal et al., 2022). These studies point to the important role played by these KLF transcription factors in the regulation of *FASN*. All these findings suggest that, consequently, rs136067046 is potentially a regulatory variant that might alter the expression of *FASN*.

To investigate the potential regulatory function of the rs136067046 variant, we constructed and then functionally tested two allele-specific recombinant promoter vectors using the dual-luciferase reporter system and the bovine mammary epithelial MAC-T cell line (Huynh et al., 1991). Details on the plasmid constructions and luciferase assays are provided in [Supplementary Material S1](#). The construct with the G allele showed a significant 2.41 ± 0.12 (mean value \pm standard deviation) fold increase (Student's *t*-test, $p = 0.0008$) of luciferase activity compared to the construct carrying the C allele ([Figure 1](#)). These results show that rs136067046 is a regulatory variant and that the G allele might lead to an increase in *FASN* mRNA synthesis by increasing its transcription rate.

In conclusion, we have demonstrated that the rs136067046 variant has a regulatory effect on *FASN* promoter activity. Further work is needed to demonstrate that it is a causal polymorphism and directly responsible for its association with milk fat traits.

AUTHOR CONTRIBUTIONS

D.R. planned and coordinated the study, analysed the results and wrote the manuscript. M.F. and M.P. prepared the allele-specific plasmids. L.B.B. and M.P. performed the luciferase assays. M.B.B. and M.C. did the bioinformatics analyses. A.B., H.T., N.D. and V.B. contributed to data analyses. All authors reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

No data availability statement is needed as no data was generated, except the luciferase measurements but it's not usually required.

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