Detection of two SNPs in exon 2 and 6 of the *LIPE* gene in high- and low-milk producing Holstein-Friesian cows

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Supplementary File

Methodological details

Genomic DNA extraction

Genomic DNA was extracted from blood samples (n = 25 animals/group) using the DNeasy Blood & Tissue DNA extraction Kits (QIAGEN, MA, USA), following the manufacturer's protocol.

For the PCR reaction, a 25 µL PCR mix was prepared. This included 12.5 µL of PCR master mix (Thermo Fisher Scientific, USA), 2 µL of DNA, 0.5 µL of each forward and reverse primer (10 µmol/µL) (as listed in Table S1), and nuclease-free water up to 25 µL. The PCR thermal cycle consisted of an initial step at 95°C for 3 minutes, followed by 35 cycles of 95°C for 1 minute, annealing at the temperature specified for each primer in Table 1 for 1 minute, and extension at 72°C for 1 minute. A final extension was performed at 72°C for 10 minutes. The specificity and quality of the PCR bands were confirmed using a 1% agarose gel.

Table S1. Primer sequences, annealing temperature, and PCR product sizes for the studied loci of the *LIPE* gene

Gene	Forward primer	Reverse primer	Ta (°C)	Size (bp)
Exon 2	GTGGAGCCAGGGGTGACCCAG	TATGTGACTGAGCCTGGTTGGGC	58	669
Exons 3–5	TCCCCACAGTTCACACCTGCCAT	TGCTCCTCAGCTCTGCGTCTGTTC	61	723
Exon 6	CAGAACAGACGCAGAGCTGAGGA	TTGTGGCTCTTTGCCCCTGCTG	61	506

Table S2. Genotype, allele frequencies, and chi-square values of the identified SNPs

	Genotype frequency						**Allele frequency**				**Chi-square**		***p* value**	
	HMY			**LMY**			**HMY**		**LMY**		**HMY**	**LMY**	***HMY***	***LMY***
LIPE 1	**CC**	**TC**	**TT**	**CC**	**TC**	**TT**	**C**	**T**	**C**	**T**	100.00	0.00	0.00	0.00
	0.00	1.00	0.00	1.00	0.00	0.00	0.50	0.50	1.00	0.00				
LIPE 3	CC	CA	AA	CC	CA	AA	C	A	C	A	2.041	36	0.360	< 0.001
	0.75	0.25	0.00	0.25	0.75	0.00	0.875	0.125	0.625	0.375				

LIPE 1, exon 2 with partial sequence from the flanking introns; LIPE3, exon 6 with partial sequence from the flanking introns. LMY, low milk yield; HMY, high milk yield.

Table S3. Association between different LIPE 2 (exons 3-5) genotypes and milk characteristics in Holstein Friesian cows

Items	Animal's production	Genotype	Mean ± SE	*p* value*
Milk yield (kg)	High milk yield	AA	3853.81 ± 82.32	< 0.001
	Low milk yield	AA	2060.68 ± 80.24	
Milk Temp. (°C)	High milk yield	AA	32.52 ± 0.31	0.001
	Low milk yield	AA	34.21 ± 0.32	
Fat (%)	High milk yield	AA	3.03 ± 0.180	0.041
	Low milk yield	AA	3.58 ± 0.184	
SNF (%)	High milk yield	AA	7.92 ± 0.167	0.023
	Low milk yield	AA	8.49 ± 0.172	
Density (g/L)	High milk yield	AA	28.01 ± 0.312	0.025
	Low milk yield	AA	29.05 ± 0.320	
Protein (%)	High milk yield	AA	2.99 ± 0.023	< 0.001
	Low milk yield	AA	3.12 ± 0.024	
Lactose (%)	High milk yield	AA	4.42 ± 0.045	0.001
	Low milk yield	AA	4.67 ± 0.049	
Ash (%)	High milk yield	AA	0.67 ± 0.005	< 0.001
	Low milk yield	AA	0.70 ± 0.005	
Milk Freezing point (°C)	High milk yield	AA	-0.51 ± 0.005	< 0.001
	Low milk yield	AA	-0.54 ± 0.005	

SNF, solids-not-fat; data are presented as least squares means ± standard errors

* *p* values denote the effect of animal groups, according to their milk yield, on milk quality.

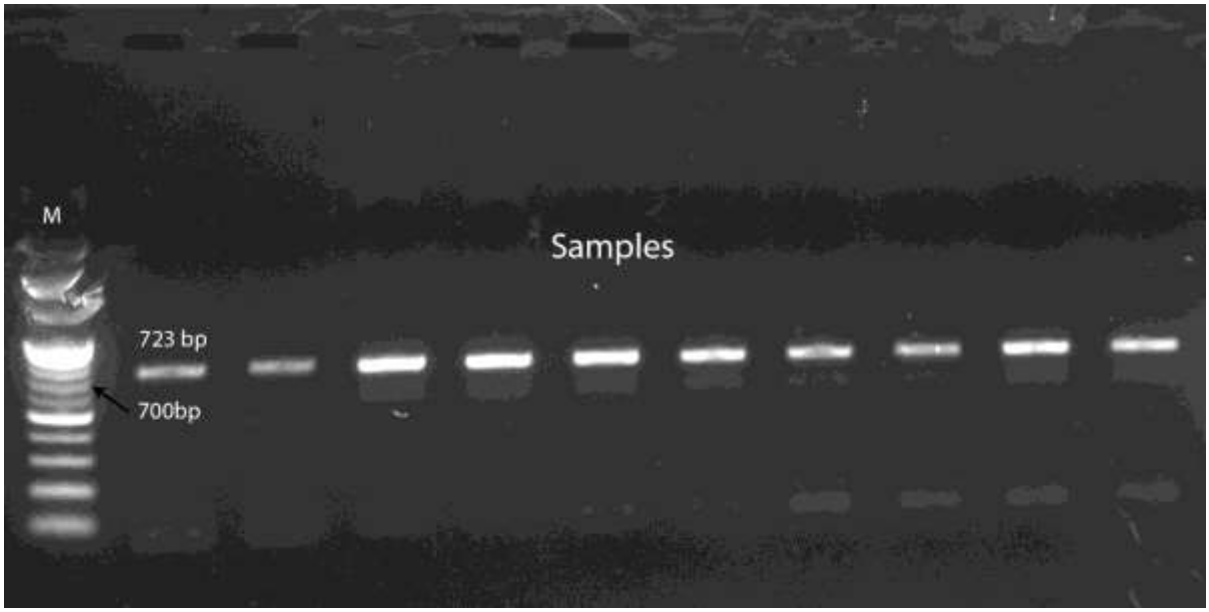

Figure S1. Ethidium bromide-stained 1.5% agarose gel of PCR products from LIPE 2 (exons 3–5 with partial sequence from the flanking introns) of the cattle *LIPE* gene (*lipase E*, hormone sensitive type); target size 723 bp. M represents the DNA marker.

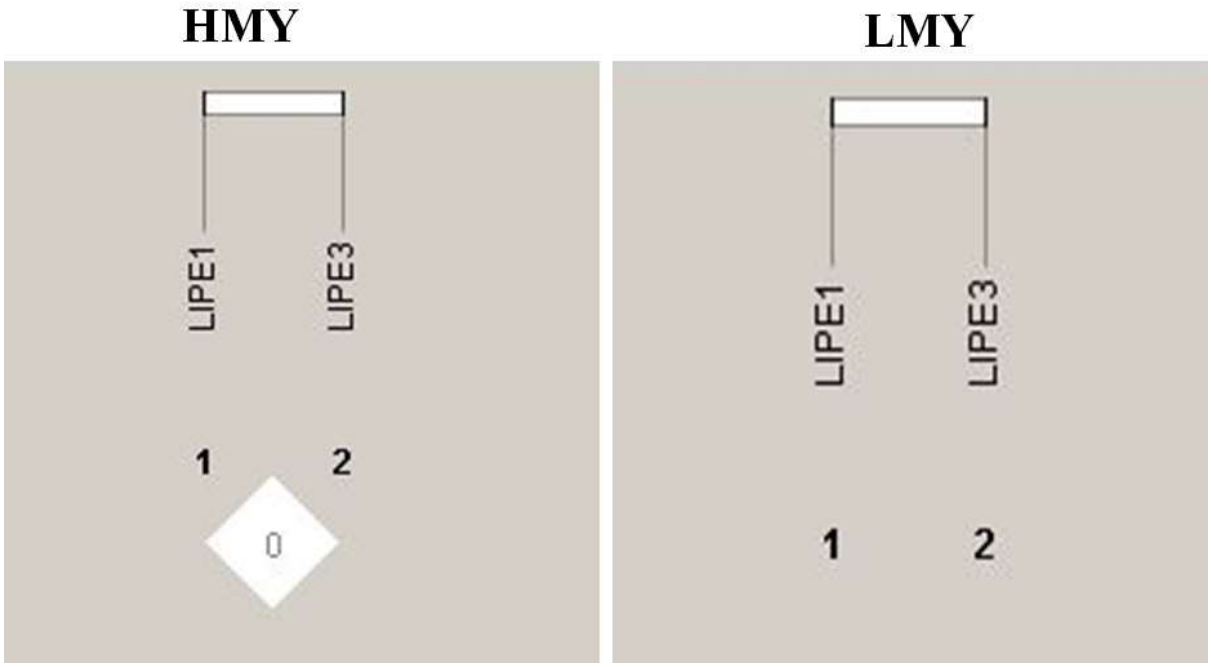

Figure S2. Pair-wise linkage disequilibrium analysis between the LIPE1 and LIPE3 SNPs in the high milk yield (HMY) and low milk yield (LMY) cows. In both studied cattle populations (HMY & LMY), the linkage disequilibrium coefficient and the absolute association were both calculated to be D' = 0.00 (0%) and r^2 = 0.00, respectively. This is visually represented in the figure by the presence of a white area and the absence of diamond formations in the HMY and LMY cows, respectively.