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Association analyses of single nucleotide polymorphisms in the *leptin* and *leptin receptor* genes on milk and morphological traits in Holstein cows

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ABSTRACT

To date, only a few SNP in the *leptin receptor* gene have been detected and analyzed while the *leptin* gene has been extensively characterized and several polymorphisms were detected and associated to functional and productive traits in cattle. In this work, for the first time, the bovine *leptin receptor* gene was fully characterized by sequencing the whole coding region and part of the 5' flanking region. A group of 95 Holstein cows was genotyped in order to search for SNP at both the *LEPR* and *LEP* genes, and to associate them with milk and morphological traits. Nine novel SNP in the *leptin receptor* gene and one novel SNP in the *leptin* gene were detected. Four SNP of the *leptin receptor* gene and one SNP of *leptin* gene showed a significant effect on one or more analyzed traits, and, in all cases, the greatest effect was observed on fat content. This study provided knowledge of the existence of further polymorphisms in the *leptin receptor* and *leptin* genes that have an influence on some important economic traits.

Keywords: Polymorphis; *Leptin* Gene; *Leptin Receptor* Gene and Dairy Traits

1. INTRODUCTION

The *leptin receptor* (*LEPR*) is a glycoprotein with a single transmembrane-spanning region; it consists of 1165 amino acids and is a member of the class I cytokine receptor family [1]. Reference [2] reviewed the activity of the *LEPR* gene, which is expressed in six isoforms, due to alternative RNA splicing. The isoforms are divided into three classes: long, short, and secretory. The long, fully active isoform (*LEPR*-b) is expressed mainly in the hypothalamus, where it takes part in the energy homeostasis and in the regulation of the activity of the secretory organs.

The bovine *LEPR* gene is located on chromosome 3q33 [3] and is composed of eighteen exons. It has only been partly sequenced and, until now, few SNP were detected. Reference [4] detected five SNP localized in exon 4 of the *LEPR* gene in some Chinese cattle breeds and, after evaluating their effects on growth traits, they suggested that this gene may be a potential candidate for growth in animals. More interesting were the findings of Liefers [5] who detected a C-T missense mutation that causes the Threonine-Methionine amino acid substitution in the intracellular domain of the *LEPR*-b isoform. These authors reported that this SNP is associated with leptin concentration during late pregnancy. The same mutation was further associated with fat and protein content in Jersey cattle, but not with milk yield [6]. Reference [7] reported only a weak association of this SNP with milk yield and days to first service, while [8] found no significant association either with milk production, or feed intake, or body energy traits, in UK dairy cows. Also reference [9] could not associate this SNP either with milk yield or composition.

The leptin hormone, a peptide of 146 amino acids, is synthesized and secreted primarily by adipose cells and by other organs and tissues, including stomach, muscle, placenta and fetal tissues [10]; it is involved in the feed intake, the energy partitioning and the metabolism of the cow [11,12]. The *leptin* gene (*LEP*) is located on chromosome 4q32 in the bovine [13] and is composed of

three exons, spanning around 18.9 kb of the genome [14]. Several polymorphism of the *LEP* have been described [15,16] and associated with serum leptin concentrations [17,18], feed intake, milk yield, energy balance and fertility [16,19], milk energy output [8,20], milk composition [9], energy storage [21,22] and growth, fertility and milk production [7]. While the *LEP* gene has been extensively characterized, and several polymorphisms were associated to functional and productive traits in cattle, only few SNP in the *LEPR* gene have been detected and analyzed. The purpose of this work was therefore to fully characterize the bovine *LEPR* gene by sequencing the whole coding region and part of 5' flanking region, in order to search for novel SNP and to evaluate the effect of the SNP of both *LEP* and *LEPR* genes on milk production and morphological traits of the same group of animals.

2. MATERIAL AND METHODS

2.1. Animals and Phenotypes

Official milk recording data, spanning over 10 years time, from 95 Holstein cows were used in this study. Cows belonged to four commercial neighboring farms located in Northern Italy, the most vocated Italian area for milk production. DNA was obtained from blood for each recorded animal. The following parameters were considered: fat and protein content at the test day; mature equivalent (ME) milk yield, fat and protein content. For 48 of these cows, the results of the morphological evaluation, performed by the Herdbook experts were available; therefore the following parameters were also included in the statistical analysis: stature, body depth, angularity, rump angle, rump width, rear legs, udder depth.

2.2. SNP Detection and Genotyping

Polymorphism detection and genotyping was performed for all samples by direct sequencing on the 3500 Genetic Analyzers (Applied Biosystems, Foster City, CA, USA). For the *LEPR* gene, 19 primer pairs were designed on the *Bos taurus* whole genome shotgun sequence (accession no. NW_001494806) and are summarized in **Table 1**. The amplified fragments include the whole coding sequence and part of the 5' flanking region. For the *LEP* gene, all cows were genotyped at the already reported SNP by [15,16,23]. Primers used and position on the gene are summarized in **Table 2**. In order to sequence the amplicons Promoter 1 (1141 bp) and Promoter 2 (613 bp) that presented a very high CG content together with several deletions and insertion it was necessary to design four more forward (P1-for1, P1-for2, P1-for3, P2-for) and two more reverse primers (P1-rev, P2-rev), that fell inside the amplicon, so to obtain shorter sequences ensuring a greater analytical specificity, without affecting the efficiency.

Table 1. Primers used for the amplification and sequencing of the bovine *LEPR* gene.

Primer	Forward primer	Reverse primer	T.a.	Size (bp)
5' flanking	5' gggattgtagggatggtcct 3'	5' ttttgcaacatggttttgga 3'	55˚C	499
exon 1	5' gcatgtgcataggaatgaacc 3'	5' ggggagggtatgtttctgga 3'	58˚C	498
exon 2	5' gcattaggcattgattgagc 3'	5' tcagaatcacatccagagacatc 3'	58˚C	578
exon 3	5' gggcacagacatatgaccaa 3'	5' ttgtgtcatgggaatgtcaa 3'	55˚C	324
exon 4	5' actgttgcctcattgtct 3'	5' taggcacaacttaccaac 3'	55˚C	473
exon 5	5' tggttctgtaattagcctttgga 3'	5' ggagggcatctcacctttc 3'	60˚C	345
exon 6	5' gacctgcttgtaatcgattttg 3'	5' gaggaagctctcatacaacttttca 3'	60˚C	401
exon 7-8	5' ctgaatgcctggtgaatcct 3'	5' tctcaccatatccacagaaaaa 3'	58˚C	708
exon 8	5' tccattgaagatgctaaggaaa 3'	5' tctttcaggaggaaagaggat 3'	58˚C	319
exon 9	5' tcttattgctttcatcattgttgt 3'	5' tcagtctgaatccaaatctgct 3'	58˚C	315
exon 10	5' aacttccatctccggcttg 3'	5' ggaggatgtatttttatgccagt 3'	58˚C	407
exon 11-12	5' tgcacctcagggtttttagc 3'	5' ccttccaaagcaaagtgaca 3'	55˚C	416
exon 13	5' tccaggagcttggcttgtat 3'	5' ggaggggtttgcaaagatta 3'	55˚C	350
exon 14	5' tctgctttctcttccttcttcc 3'	5' tttttgaagtttccattaactgtg 3'	60˚C	389
exon 15	5' aattcagtagggatgactctttaaaat 3'	5' gacaccatggaaccatataccc 3'	64˚C	311
exon 16	5' cgatgaaatcagaaaagggtatg 3'	5' tgcagtgtggattcctgaaa 3'	58˚C	353
exon 17	5' tgtgatggcatatgaaaggaa 3'	5' caaaaatgtttgaagttctaaaagg 3'	58˚C	303
exon 18a	5' cacacttccatttcttccatga 3'	5' tggtggagaattgttgctca 3'	58˚C	485
exon 18b	5' ggtgaaactgaggaggagca 3'	5' tgttttcttttctactctcctaacca 3'	63˚C	607

Table 2. Primers used for the amplification and sequencing of the bovine *LEP* gene.

Primer	Forward primer	Riverse primer	T.a	Size (bp)
Promoter 1	5' aggcggagaggaggaaagat 3'	5' atgatggtgtggaggggtaa 3'	56˚C	1141
P1-for 1	5' acatttctttatttgacagttccag 3'			
P1 for 2	5' tagtacaatatccttcctttctt 3'			
P1 for 3	5' tgtgatcagaaaacacataccatttta 3'			
P1 rew		5' catagcagcaatatgcacaca 3'		
Promoter 2	5' cacgttcccgttaggaagtctctg 3'	5' cggcggagaagtagaaaggagaga 3'	50˚C	613
P2 for	5' cttacccctccacaccatcatcaa 3'			
P2 rew		5’ cgattcctcgatccctccg 3'		
Exon 2	5' ccagggagtgcctttcatta 3'	5' atggccacggttctacctc 3'	57˚C	395
Exon 3	5' ccctctctcccactgagctc 3'	5' taaaggatgcccacataggc 3'	57˚C	496

2.3. Statistical Analysis

The allele substitution effect of each SNP was estimated, similarly to [24,25], by regressing the number of copies of each allele against each of the traits separately, using the following Linear Mixed Model in SAS [26]:

$$Y_{ijklm} = \mu + B_i + + C_j + D_k + G_l + A_m + e_{ijklmn}$$

where: Y_{ijklm} = Phenotype[1]; μ = overall mean; B_i = fixed effect of the herd; C_j = fixed effect of the season/year of calving; D_k = fixed effect of the age at calving; G_l = allele substitution effect; A_m = random animal effect; e_{ijklmn} = residual.

A GLM that included only the allele substitution effect as covariate was used for the ME milk yield, fat and protein content, because these parameters were already corrected for the fixed effects. A GLM that included the year of birth and the age at the evaluation as fixed effects was used for the morphological indexes.

A false discovery rate (FDR) was also calculated using PROC MULTTEST in SAS [26] to account for multiple testing, whereby significance was achieved at an adjusted FDR of $P < 0.10$, as suggested in [7]. Linkage disequilibrium (LD) between loci was estimated using PROC ALLELE in SAS [26] in order to evaluate the pair-wise correlation coefficients for all SNP within each of the two genes, so to avoid false-positive effects and not to ascribe to a SNP the effect of another one, in case the alleles of the second were highly correlated with the first.

2.4. Transcription Factor Binding Sites

The MATCH program [27] in the TRANSFAC® Professional 10.2 http://www.biobaseinternational.com/ [28] was used to perform the profile search for the transcription factor binding sites for the two genes. The vertebrate binding matrices and the only high quality matrix option were used in the profile selection, and the cut-offs for core and matrix similarity were set to 0.999 and 0.7 respectively, with no further change in the other options.

[1]Test day milk fat percentage, test day milk protein percentage.

3. RESULTS

In **Tables 3-4**, the position in the gene and the minor allele frequency (MAF) of the detected SNP in the analyzed population, for the *LEPR* and the *LEP* gene was reported. Sequence analysis of the DNA of the 95 Holstein cows, across the 7.8 kb region of the bovine *LEPR* gene, that includes part of the 5’ flanking region and the whole coding region, revealed a total of 16 SNP; 9 of them being novel detected SNP, not previously referred in the literature (**Table 3**). The novel SNP LEPR01 and LEPR02 are located in the 5' flanking region; LEPR04, LEPR10 and LEPR16 are synonymous mutations, located, respectively, in exons 7, 12, and 18; LEPR06 is a missense mutation in exon 8 that causes the amino acid substitution S460G at the position 460 of the protein; LEPR09 is also a missense mutation in exon 11, producing the amino acid substitution R615S at the position 615 of the protein. Because [29] showed that the region from 428 to 635 of the protein includes the binding sites for the leptin, the two missense mutations may be fundamental for binding and activate the leptin hormone. Finally, LEPR11 and LEPR14 are located in the introns 15 and 17.

Sequence analysis of the DNA of the 95 Holstein cows, across the 2.6 kb region of the bovine *LEP* gene, revealed a total of 26 SNP (24 in the promoter and 2 in the exons). Of the 26 detected SNP, 19 had already been reported by [16] (accession no. AJ571671); three SNP were reported in the dbSNP (rs29004171; rs29004172; rs29004173); one SNP (accession no. AB070368) had

been previously reported by [23] and two SNP (rs29004488 and rs29004508) had been reported by [15]. Finally, a novel SNP, LEP05, was detected in the present study (g.2003435T>C; accession no. NW_001494939).

Table 3. Analyzed SNP in the *LEPR* gene.

SNP	GenBank position NW_001494806	refSNP	Position in the gene	Type of mutation	Protein position	Minor allele	MAF %
LEPR01	g.134260C > T	new	5' flanking region			T	0.18
LEPR02	g.134261G > C	new	5' flanking region			C	0.10
LEPR03	g.138780T > G	rs43347906	Exon 2	missense	L35V	T	0.46
LEPR04	g. 177514G > T	new	Exon 7	cds-synon	A343	T	0.18
LEPR05	g. 178811C > T	rs43208611	Intron 7	intronic		C	0.40
LEPR06	g.178022A > G	new	Exon 8	missense	S460G	G	0.17
LEPR07	g.178107G > A	rs43208612	Intron 8	intronic		G	0.35
LEPR08	g.183395A > G	rs43349293	Exon 10	cds-synon	P561	A	0.08
LEPR09	g.184626G > C	new	Exon 11	missense	R615S	C	0.09
LEPR10	g.184818C > T	new	Exon 12	cds-synon	I640	T	0.18
LEPR11	g.195298C > A	new	Intron 15	intronic		C	0.18
LEPR12	g.195382C > T	rs43349279	Exon 16	cds-synon	I844	C	0.36
LEPR13	g.196244C > T	rs43349276	Intron 16	intronic		T	0.29
LEPR14	g.196384A > G	new	Intron 17	intronic		G	0.20
LEPR15	g.209779C > T	rs133672995	Exon 18	missense	T944M	T	0.10
LEPR16	g.210413A > C	new	Exon 18	cds-synon	I1155	C	0.07

Table 4. Analyzed SNP in the *LEP* gene.

SNP	GenBank position NW_001494939	refSNP or accession	Position in the gene	Type of mutation	Protein position	Minor allele	MAF %
LEP01	g.2003314 G > A	AJ571671 g.143	Promoter			A	0.42
LEP02	g.2003319 G > A	AJ571671 g.146	Promoter			A	0.42
LEP03	g.2003325 C > T	AJ571671 g.154	Promoter			T	0.42
LEP04	g.2003379 A > G	rs109337813	Promoter			A	0.40
LEP05	g.2003435 T > C	new	Promoter			C	0.12
LEP06	g.2003516_2003517 ins AG	AJ571671 g.344..47	Promoter			no ins	0.40
LEP07	g.2003531 C > G	rs137564647	Promoter			C	0.40
LEP08	g.2003707_2003711 del GT	AB070368g.1935..37	Promoter			del	0.42
LEP09	g.2003806 T > C	rs109956568	Promoter			T	0.37
LEP10	g.2003868 T > A	rs29004468	Promoter			T	0.37
LEP11	g.2004191 G > C	rs29004170	Promoter			C	0.42
LEP12	g.2004196 G > A	rs29004171	Promoter			A	0.12
LEP13	g.2004270_2004271 ins C	rs29004172	Promoter			ins	0.19
LEP14	g.2004286 G > A	rs29004173	Promoter			A	0.19
LEP15	g.2004356_2004357 del G	rs29004469	Promoter			del	0.42
LEP16	g.2004478 C > T	rs29004470	Promoter			T	0.42
LEP17	g.2004488 G > T	rs29004471	Promoter			T	0.19
LEP18	g.2004498 A > G	rs29004472	Promoter			A	0.40
LEP19	g.2004559 A > G	rs29004473	Promoter			G	0.19
LEP20	g.2004569 C > T	rs29004474	Promoter			T	0.19
LEP21	g.2004573 A > C	AJ571671 g.1403	Promoter			C	0.00
LEP22	g.2004600 T > C	rs29004475	Promoter			T	0.37
LEP23	g.2004623 T > C	rs29004476	Promoter			C	0.42
LEP24	g.2004665 G > C	rs29004477	Promoter			G	0.40
LEP25	g.2016952 T > C	rs29004488	Exon 2	missense	R25C	T	0.40
LEP26	g.2018875 C > T	rs29004508	Exon 3	missense	A80V	T	0.22

In this study, the *in silico* analysis with the MATCH software was performed to verify whether the SNP that resulted significantly associated to productive traits, were located in putative binding sites of transcription factors. The results of this analysis indicated that LEP14 is located at the putative binding site for v-Maf and NF-E2 transcription factors and that the A allele does not activate the binding sites of either of the two.

In the association analysis, of the 16 SNP of the *LEPR* gene, the following 6 were used: LEPR01 and LEPR02 because located in the promoter; LEPR03, LEPR09 and LEP15, because they encode a missense mutation; LEPR16 because of its short distance (30 bp) from the 3'UTR. Similarly, of the 26 SNP of the *LEP* gene, the following 4 were considered in the association analysis: LEP01 because of the significant associations reported by [7] on growth and fertility; LEP14, because of its position within the binding site of transcription factors (v-Maf and NF-E2), LEP25 and LEP26 because they encode a missense mutations.

In **Table 5** and **Table 6**, the results of the association analysis are presented only for those SNP of *LEP* and *LEPR* that showed statistically significant effects, reporting also the effects of the same SNP on other traits, if trending to statistical significance ($P < 0.09$). **Table 5** describes the association of 5 SNP with milk traits. Mean values and standard deviation for each trait are reported in the table, so to allow a direct evaluation of the extent of the allelic substitution effect. All these values fall in the range of the average production traits of Holstein cows in Italy.

Three SNP of the *LEPR* gene (LEPR03, LEPR09, and LEPR16) influenced ME fat content, with substitution effects ranging from 0.11 ($P = 0.04$) to 0.26 ($P = 0.007$); LEPR16 influenced also the test day fat content (0.38; $P = 0.02$). One SNP of *LEP* gene (LEP14) influenced the test day fat content (0.19; $P = 0.02$), and also showed a trend to influence ME fat content (0.10; $P = 0.09$). Furthermore, three SNP of the *LEPR* gene (LEPR01, LEPR09 and LEPR16) influenced protein content, with substitution effects ranging from 0.08 ($P = 0.04$) to 0.11 ($P = 0.03$) for ME protein content, and from 0.10 ($P = 0.02$) to 0.11 ($P = 0.07$) for the test day protein content.

Table 5. Allele substitution effect of the SNP in the *LEP* and *LEPR* genes on milk production traits: mature equivalent (ME) and test day.

SNP	Trait/allele	Position in the gene	ME milk yield (kg)	ME fat (%)	ME protein (%)	Test day fat (%)	Test day protein (%)
Mean			9607.58	3.59	3.24	3.77	3.50
St dev			1870.84	0.46	0.23	0.92	0.50
n. cows			77	77	77	95	95
n. lactations			184	184	184	225	225
LEP14	G	Promoter		0.10 $P = 0.09$		0.19 $P^* = 0.02$	
LEPR01	C	5' flanking			0.08 $P^* = 0.04$		0.10 $P^* = 0.02$
LEPR03	G	Ex 2		0.11 $P = 0.04$			
LEPR09	G	Ex 11		0.26 $P^* = 0.007$	0.09 $P^* = 0.05$		0.11 $P^* = 0.03$
LEPR16	A	Ex 18		0.26 $P^* = 0.02$	0.11 $P^* = 0.03$	0.38 $P = 0.02$	0.11 $P = 0.07$

*Significant after correction for false discovery rate (FDR, $P < 0.10$).

Table 6. Allele substitution effect of the SNP in the *LEP* and *LEPR* genes on morphological indexes expressed in points (pts). The effect refers to the allele of column 2.

SNP	Allele/Trait	Position in the gene	Stature	Body depth	Angularity	Rump width	Rear legs
Mean			30.5	27.7	25.2	26.8	20.9
St dev			8.3	8.3	6.8	8.1	6.8
n. cows			48	48	48	48	48
LEP01	A	Promoter				4.3 $P = 0.03$	5.3 $P^* = 0.002$
LEP25	C	Ex 2		3.7 $P = 0.05$			
LEPR01	C	Promoter			5.1 $P = 0.05$		4.7 $P = 0.09$
LEPR03	G	Ex 2	–4.2 $P = 0.05$				
LEPR16	A	Ex 18			6.5 $P = 0.05$		

*Significant after correction for false discovery rate (FDR, $P < 0.10$).

In **Table 6**, the results of the association analysis between 5 SNP of the *LEP* and *LEPR* genes and morphological indexes, expressed in points (pts) are shown. These traits were included in this study under the hypothesis that the genes that regulate the fat storage physiology, like the *LEP* and *LEPR* genes, might affect some morphological traits; similarly, Waters [30] quantified the associations of some SNP in the genes of the somatotrophic axis with milk performance and conformation traits in Holstein cows. Three SNP of the *LEPR* gene (LEPR01, LEPR03, and LEPR16) showed a significant effect on either the angularity or the stature or the rear legs (**Table 6**). Only one SNP of the *LEP* gene (LEP01) affected rump width and rear legs with values respectively of 4.3 pts and 5.3 pts. None of the analyzed SNP affected significantly either the rump angle or the udder depth. In **Table 7** and **Table 8**, separately for the SNP of the *LEPR* and the *LEP* genes, in order not to ascribe to a SNP the effect of another one, for the SNP that showed some effect on any of the traits, pair-wise allelic correlations were reported.

4. DISCUSSION

These results showed that fat content is the most affected trait by the *LEPR* and *LEP* gene polymorphisms, while protein content is significantly affected only by *EPR* gene polymorphisms. As regards to the *LEPR* gene, two SNP, LEPR09 and LEPR16, influence both fat and protein (**Table 5**). LEPR09 encodes a missense mutation in exon 11 causing the amino acids substitution R615S at residue 615. The Arginine is a polar and basic amino acid while the Serine is polar and neutral; moreover, the substitution falls in the extracellular domain, five residuals before the starting of the second W-S-X-W-S motif. Reference [31] reported that the W-S-X-W-S motifs are involved in the ligand binding. It is then possible to justify the role played by LEPR09 in the variation of fat and protein content. Also LEPR16 affects both parameters, but because it encodes a synonymous mutation, it is likely that such effect be due to the high pair-wise correlations with LEPR09 (0.87; $P < 0.0001$; **Table 7**). LEPR03 influenced only fat content; this SNP encodes the missense mutation, in exon 2, causing the amino acid substitution L35V at residue 35, this substitution falls in the extracellular region of LEPR molecule very near the putative signal peptide region. The evidence that the considered SNP affect particularly milk fat is corroborated by the function of the two genes in lipid regulation; however, because the considered animal sample was relatively small, the results should be regarded as an indication of the possible association of these SNP with the considered traits, that need to be further confirmed in independent dairy populations.

The results of this work indicated that also LEPR01 affected significantly protein content but the effect, very similar to the effect of LEPR09, is likely due to the partial and highly significant correlation between the two variants (**Table 7**).

It is interesting to note that the majority of the authors, that have performed association studies on the *LEPR* gene, have analyzed only the SNP LEPR15 (rs133672995) which encodes the missense mutation Threonine to Metionine at the 944 residue of the protein sequence (accession no. NP_001012285). Reference [5] found that this SNP significantly influenced blood concentration of circulating leptin; however, no significant association of this SNP with milk-related traits was found [8,32], in agreement with our results.

Table 7. Allelic correlations between the pairs of SNP of the *LEPR* gene that were included in the association analysis.

SNP 1/SNP 2	LEPR02	LEPR03	LEPR09	LEPR12	LEPR16
LEPR01	0.21 $P = 0.001$	0.38 $P = 0.0001$	0.61 $P = 0.0001$	0.09 *ns*	0.49 $P = 0.0001$
LEPR02		0.46 $P = 0.0001$	0.11 *ns*	0.53 $P = 0.0001$	0.05 *ns*
LEPR03			0.21 $P = 0.002$	0.72 $P = 0.0001$	0.16 $P = 0.01$
LEPR09				0.29 $P = 0.005$	0.87 $P = 0.0001$
LEPR12					0.25 $P = 0.002$

Table 8. Allelic correlations between the pairs of SNP of the *LEP* gene that were included in the association analysis.

SNP 1/SNP 2	LEP14	LEP25	LEP26
LEP01	0.37 $P = 0.0001$	0.60 $P = 0.0001$	0.27 $P = 0.04$
LEP14		0.26 $P = 0.006$	0.20 $P = 0.04$
LEP25			0.16 *ns*

Of the SNP of the *LEP* gene, only LEP14 showed significant effects on some of the considered traits. On the contrary, a significant effect of LEP25 on total milk yield was proposed by [19], while [16] found a significant effect of LEP09 on fat yield, and of LEP11 on protein content. Reference [33] showed that LEP09 and LEP25 were significantly associated with milk fat and protein content, while LEP26 was associated only with the survival in the herd. Reference [9] reported a significant effect of LEP01 and LEP09 with milk protein content. Finally, [7] investigated the associations between the SNP in the *LEP* and *LEPR* genes, with growth, milk production, and fertility traits and found that LEP01 was significantly associated with height at withers and with the total number of AI services in heifers, while LEP26 was significantly associated with crown rump length, age at first service, at daily milk yield and 305-d milk yield.

It is interesting to note that LEP14 falls in the core sequence of an important binding site of transcription factors: NF-E2 (gGCTGActttc). The NF-E2 is involved in the regulation of globin gene transcription [34] and shows a high degree of homology to the Nrf2 transcription factor [35] that plays a role in lipid metabolism [36, 37]. In fact, [38] reported that the expression of many genes, that are involved in the synthesis and catabolism of cholesterol and fatty acids, is inversely related to the amount of Nrf2. The Nrf2 might also modulate leptin expression, by interfering with lipogenic pathways, so corroborating the positive effect of allele G of LEP14 on milk fat content.

As regards to the association between the SNP and the morphology, some SNP of the *LEPR* gene seemed to influence both milk and morphological traits. In fact, the trait angularity, that summarizes the ideal dairy cow type, is positively influenced by the A allele of LEPR16, that showed a positive effect on both fat and protein content (**Table 5**), confirming that milk traits are positively correlated with the morphological parameters desired for the dairy cow type.

As regards to the *LEP* gene, we noted that the desired morphological parameters for the dairy cow (body depth, rump width and rear legs) are positively influenced by LEP01 and LEP25 (**Table 6**), and these SNP are also highly correlated (**Table 8**).

5. CONCLUSION

In this study, for the first time, the bovine *LEPR* gene was fully characterized by sequencing the whole coding region and part of 5'flanking region and several novel SNP were detected, some of which encode missense mutations; moreover, a novel SNP was detected in the promoter of the *LEP* gene. In this study, we described 26 SNP of the *LEP* gene and 16 SNP of the *LEPR* gene and we reported significant associations of some of them with important milk production and morphological traits. Previous authors had aimed to estimate the *LEP* and *LEPR* gene effects on milk production traits, but because only one or few SNP were considered in each study, the results were contrasting and difficult to be extended to other cow populations. To our knowledge, this is the first study that evaluated the effect of many SNP of the *LEPR* gene on dairy traits. Due to relatively small size of the considered animal sample, the detected effects need to be further confirmed in independent dairy populations; however, because the allele substitution effects of the SNP of both the genes were evident, in most cases, on fat content, the results of this study confirm the role that both genes play in influencing this trait.

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