Spanish Journal of Agricultural Research
14(4), e04SC02, 5 pages (2016)
eISSN: 2171-9292
http://dx.doi.org/10.5424/sjar/2016144-9279
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

SHORT COMMUNICATION

OPEN ACCESS

Analysis of polymorphisms in candidate's genes for meat quality in Lidia cattle

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Abstract

The aim of this paper was to analyze the segregation of some polymorphisms in three genes (*CAPN1*, *CAST* and *DGAT1*) related to meat quality in the Lidia cattle breed and some of its main lineages. To that effect, 119 individuals from the Lidia breed were analyzed. Although the association between the polymorphisms and the phenotype has never been demonstrated in this breed, the absence of fixed genotypes for these polymorphisms in the studied population makes the Lidia cattle a good candidate to develop selection objectives. The clear differentiation among lineages for most of the genes studied reinforces the high reproductive isolation presented in the Lidia cattle as revealed by previous studies on the structure of the population within the Lidia breed using microsatellite markers. Considering both issues in the design of breeding schemes will be necessary to save the lineages and not to lose this valuable genetic resource. Finally, it would be necessary to carry out an in depth search for new polymorphisms in genes associated with meat quality and to perform needed association analyses between the SNPs segregating in Lidia cattle and traits of economic interest.

Additional key words: bullfighting; calpastatin; single nucleotide polymorphisms.

Abbreviations used: MNA (mean number of alleles per locus); SNP (single nucleotide polymorphisms).

Authors' contributions: Conceived and designed the experiments, and critical revision of the manuscript for important intellectual content: MV and AM. Performed the experiments: RP. Analyzed the data and wrote the paper: RP and CA.

Citation: Pelayo, R.; Valera, M.; Molina, A.; Avilés, C. B. (2016). Short communication: Analysis of polymorphisms in candidate's genes for meat quality in Lidia cattle. Spanish Journal of Agricultural Research, Volume 14, Issue 4, e04SC02. http://dx.doi.org/10.5424/sjar/2016144-9279.

Supplementary material (Figs. S1, S2, S3, S4 and S5) accompanies the paper on SJAR's website.

Received: 11 Jan 2016. **Accepted**: 11 Nov 2016.

Funding: Lidia cattle breed association ("Agrupación Española de Ganaderos de Reses Bravas").

Competing interests: The authors have declared that no competing interests exist.

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Introduction

The Lidia cattle breed is one of the most important Iberian breeds reared in the traditional agro-forestry system called "dehesa". Many aspects of the breed promote great diversity, for example, the different cultural shows, that require the selection of bulls with different behavior and morphological characteristics (Pelayo *et al.*, 2015). This fact and the extreme reproductive isolation of some farms prompted a fragmentation of the population into subpopulations, traditionally called lineages with varying levels of gene flow among them (Cañon *et al.*, 2008).

Most of the efforts made in the breeding of these cattle are focused on the aggressiveness of the bull and animals without these behavioural features are discarded. In these cases, the economic value and income from the meat is essential for the producer and breeder of Lidia cattle. The meat from excitable animals has lower quality than more docile animals as assessed by behavioral tests (Haskell *et al.*, 2014). An interesting strategy to enhance the profitability of the farm would be to assess the possibility of improving the characteristics of the meat, to widen the selection criteria of the Lidia cattle breed and look for alternative uses of a breed mainly reared in extensive systems which could provide an organic and healthy dietary source. Given the fact that sensory and nutritional quality of meat are becoming more and more important, different studies related to this topic have recently been carried out on Lidia cattle (Beriain *et al.*, 2011; Horcada-Ibáñez *et al.*, 2012). These authors characterized this breed from its

phenotypic make up. Conversely, no studies have been performed to determine the heritability of these traits, but limited to behavior trait studies (Pelayo *et al.*, 2016). Polymorphisms in genes associated with meat quality are one example of technology used to predict traits like tenderness or marbling. However, these associations must be validated in each particular breed.

The calpain-calpastatin complex (*CAPN1* and *CAST*) is an endogenous, calcium-dependent proteinase system (Goll *et al.*, 2003) involved in the *post-mortem* tenderization process since they regulate *post-mortem* proteolysis. The *CAPN1* gene has been mapped to chromosome 29 and several single nucleotide polymorphisms (SNPs) in this gene have been reported to be associated with meat tenderness in beef cattle (Page *et al.*, 2004), two of which produce glycine to alanine and valine to isoleucine substitutions in exons 9 and 14, respectively. According to Avilés *et al*. (2013) and Calvo *et al.* (2014) and the effect of the SNPs of the *CAST* on instrumental meat tenderness is evident in different beef populations reared in Spain. The diacylglycerol O-acyltransferase 1 (*DGAT1*) is a microsomal enzyme that catalyzes the final step of triglyceride synthesis (Winter *et al.*, 2002). This gene has been mapped to chromosome 14. A lysine to alanine substitution (K232A) in the *DGAT1* gene has been associated with increased milk yield and milk fat content in dairy cattle (Casas *et al.*, 2005). The aim of this paper was to analyze the frequencies of several polymorphisms in three genes related to meat quality (*CAPN1* and *CAST)* for tenderness and intramuscular fat, back fat thickness or sensory flavor and juiciness (*DGAT1*), in the whole population of Lidia cattle as well as in the main lineages currently being reared.

Material and methods

Blood samples of 119 individuals from the Lidia breed (79 males and 40 females), from 5 different lineages were analyzed (28 Domecq, 22 Marqués de Albaserrada, 24 Murube Urquijo, 20 Núñez and 25 Santa Coloma). Genomic DNA was obtained from 200 µL of whole blood using the extraction protocol of the Dominion ® MBL kit following the manufacturer's instructions. Four different DNA fragments were analyzed. A set of PCR primers were designed in two regions (*CAPN1* segment 1, from intron 8 to intron 9 and *CAPN1* segment 2, from exon 11 to exon 22) of the *CAPN1* gene (GenBank Acc. No. AH009246), the *CAST* gene (AY008267), and *DGAT1* gene (JQ897351-53). The primers used in the analyses are shown in Table 1. PCR reactions were carried out in a thermocycler (Eppendorf® AG, Germany) in a total volume of 25 µL containing 2 µL (80 ng) of bovine genomic DNA, 2.5 µL of PCR buffer, 0.75 µl of $MgCl_2$ (50 mM), 1.2 µL of dNTPs (4µM), 2 µL of each primer (5 mM), 0.1 µL of Taq DNA polymerase (5U/µL) and 14.45 µL of MQ H_2O. The thermal profiling consisted of a hot start step at 96 °C for 3 min, followed by 40 cycles of 30 s at 96 °C, 30 s at the annealing temperature of 72 °C (all genes), 4 min at 72 °C and a final extension step of 10 min at 72 °C. The obtained amplicons were purified and visualized on 2% agarose electrophoresis gels stained with ethidium bromide. Segments 1 and 2 of the *CAPN1* gene, *CAST* and *DGAT1* genes were sequenced. All sequences were edited, assembled and aligned using the program Sequencher v.4.6 software (Gene Codes Corporation). After alignment, polymorphic sites were determined and different genotypes assigned by visual examination of the electropherograms (Fig. S1 [suppl.]). The variability parameters across loci and populations for the whole population and for each different lineage were computed using the Genetix 4.2 program (Belkhir *et al.*, 2004). Fisher's exact test was performed to assess the possible association between the frequency distribution of the different polymorphisms studied and the five lineages of our population (Statistica v.6.0 software).

Results and discussion

Allele frequencies for each polymorphic locus are presented in Table 2. Six SNPs that were previously reported (Avilés *et al.*, 2009) were found in the *CAPN1* segment 1. According to published research, the *C* allele from the *CAPN1*: Ex9-g. 316 C>G was associated

Table 1. Amplified fragments and primers used in the analysis.

DNA fragments	Chromosome	Length of the fragments (bp)	Forward primers	Reverse primers
CAPN1 segment 1	BTA29	669	CGGGTGAGGGTCCATGGAGGCTG	GGTGTTCCAGTTGCGGAACCTCTGGCT
CAPN1 segment 2	BTA29	765	TCCGAAGGGTGGGCTGAGCTGC	AGCCCAATGATGAGGGGTGAGCCTG
CAST	BTA7	270	CGGCACCTCTGTGTGGCATCAGCA	GCTTGGGTAGGCTTTTTGGCTGAAAACACG
DGAT	BTA14	727	TCCCACAGTGGGCTCCGTGCTG	GCCAGGCCTGCCTGCTCACCTTG

Table 2. Allele frequencies for the identified SNPs in *CAPN1*, *CAST* and *DGAT1* genes and Fisher's exact association test with the five lineages of Lidia cattle breed. In parenthesis, number of samples of each lineage.

Genes (loci)[1]	Allele[2]	Domecq (28)	Marqués de Albaserrada (22)	Murube Urquijo (24)	Núñez (20)	Santa Coloma (25)	Overall population (119)	Fisher's test[3]
CAPN1 segment 1								
In8-g. 80 C>T	C	0.37	0.80	0.59	0.66	0.39	0.63	19.33*
In8-g. 302 C>G	C	0.45	0.94	0.70	0.75	0.54	0.64	15.11 ns
In8-g. 310 A>G	A	0.56	0.14	0.32	0.21	0.46	0.38	12.79 ns
Ex9-g. 316 C>G	C	0.71	0.14	0.36	0.29	0.63	0.45	20.01*
In9-g. 445 C>T	C	0.28	0.71	0.60	0.64	0.10	0.49	21.49*
Ex10-g. 524 A>C	A	0.25	1.00	0.80	1.00	0.00	0.64	11.01*
CAPN1 segment 2								
In13-g. 4506 C>G	C	0.50	0.83	0.56	0.82	0.37	0.58	23.60***
Ex14-g. 4558 G>A	G	0.90	1.00	0.90	0.94	0.98	0.94	11.04 ns
In14-g. 4685 C>T	C	0.82	0.50	0.58	0.79	0.73	0.72	10.72 ns
CAST								
In8-g. 282 C>G	C	0.48	1.00	0.46	0.58	0.60	0.57	35.30***
DGAT1								
Ex8-g. 232 AAAA>GCGC	AAAA	0.04	1.00	0.14	0.07	0.58	0.28	35.70***

[1] Nucleotide positions are deduced from reference sequences EU386166-83 for *CAPN1* segment 1, AF248054 *CAPN1* segment 2, AY008267 for *CAST* and JQ897351-53 for *DGAT1*. [2] "Allele" shows the allele that is reported to have positive effect on tenderness (decreases shearforce). [3] *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: no significant differences

with lower shear force values (Page *et al.*, 2004; Schenkel *et al.*, 2006). Highlighted differences between lineages for this SNP were found in the Lidia cattle breed. The *C* allele frequency ranged from 0.71 to 0.14 for Domecq and Marqués de Albaserrada lineages, respectively. Overall, the population in the Lidia cattle breed presented a frequency of the *C* allele of 0.45. Avilés *et al.* (2009) found in the Spanish maternal beef breeds frequencies ranging from 0.11 (Avileña breed) to 0.29 (Retinta breed) while Van Eenennaam *et al.* (2007), showed in European continental breeds, frequencies between 0.05 for Charolais breed and 0.13 for Simmental breed. Three SNPs were detected in the *CAPN1* segment 2, two of which were not published before: In13-g. 4506 C>G and In14-g. 4685 C>T, see Figs. S1-S5 [suppl.]. Page *et al.* (2004) associated the *G* allele from *CAPN1*: Ex14-g. 4558 G>A with lower shear force values. The *G* allele frequency ranged between 1.00 in Marqués de Albaserrada lineage to 0.90 in Domecq and Murube Urquijo lineages. Overall, the frequency of the *G* allele for the population in the Lidia cattle breed is 0.94, while Allais *et al.* (2011) published frequencies in European continental breeds ranging from 0.76 (Charolais breed) to 0.64 (Limousin breed). However, it would be interesting to perform an in depth study of the two new markers; *CAPN1*: In13-g. 4506 C>G and In14-g. 4685 C>T. Frequencies for the *C* allele from the *CAST*: In8-g. 282 C>G SNP ranged between 1.00 in Marqués de Albaserrada lineage to 0.46 in Murube Urquijo lineage. Overall, the population presented a frequency of 0.57 of the allele associated with tender meat (Schenkel *et al.*, 2006). Van Eenennaam *et al.* (2007) found frequencies for the *C* allele ranging from 0.63 (Angus breed) to 0.73 (Limousin breed). Finally, remarkable differences were found in Lidia cattle between lineages for *DGAT1*: Ex8-g. 232 AAAA>GCGC. The lysine variant of the marker has been associated with a higher lipid content in different tissues (Thaller *et al.*, 2003; Avilés *et al.*, 2013). In our population, the lysine allele frequency ranged from 1.00 in Marqués de Albaserrada lineage to 0.04 in Domecq lineage. Overall, the population in Lidia cattle breed presented low frequency of the *KK* genotype (0.28). This result was consistent with those published in Lidia cattle breed (0.21) by Kaupe *et al.* (2004).

Our results showed that there are statistically significant differences in the distribution of frequencies of the studied markers and the different lineages. The genetic variability was low in all lineages (Table 3) which is partitioned into very few farms that impose a high reproductive isolation, with a higher heterozygosity in the Murube Urquijo lineage (0.409) and a lower heterozygosity (0.197) in the Marqués de Albaserrada lineage (in accordance with the frequencies mentioned previously). This higher reproductive isolation of all the Lidia lineages (with regards to other cattle breeds) imposed by breeders with the objective to maintain their branded features (Sanz *et al.*, 2014) may be the cause of the heterozygosity deficit observed in the five lineages. The effective size within the Lidia cattle lineages is very low. Therefore, it

Table 3. Expected (He) and observed (Ho) heterozygosity and mean number of alleles per locus (MNA) values in the five *lineages* of Lidia breed for the *CAPN1*, *CAST* and *DGAT1* genes analysed. Standard deviation in parenthesis.

Lineage	He	Ho	MNA
Domecq	0.380 (0.146)	0.267 (0.189)	2
Marqués de Albaserrada	0.197 (0.187)	0.167 (0.212)	1.636
Murube Urquijo	0.409 (0.109)	0.310 (0.245)	2
Núñez	0.313 (0.190)	0.300 (0.258)	1.818
Santa Coloma	0.364 (0.193)	0.251 (0.251)	2
Overall population	0.430 (0.110)	0.274 (0.207)	2.091

is impossible to find unrelated animals within different lineages. The mean number of alleles (MNA) per lineage varied from 2 in Domecq, Murube Urquijo and Santa Coloma lineages to 1.636 in Marqués de Albaserrada, with a mean for the overall population of 2.091.

The results of this study in the Lidia cattle breed show the frequency of the alleles previously associated with more tender meat and higher fat content. Finally, it would be interesting to carry out an in depth search for new polymorphisms in genes associated with meat quality and the need of performing association analyses between the SNPs segregating in Lidia cattle and traits of economic interest.

Acknowledgements

We acknowledge the collaboration of the Lidia Cattle Breed Association (AEGRB).

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