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Detection of polymorphisms in the cast gene from brangus and simmental cattles using next generation sequencing (#210)

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Introduction

Nutritional and environmental factors as well as genetic factors and genomic composition are known to have a significant effect on meat quality. The potential of genomic data to increase the quality of meat has been an emphasized subject for many years. The effects of genetic variations on meat tenderness and textural properties of meat have been demonstrated by several studies revealing that the polymorphisms in the calpastatin (CAST) gene play an important role in determining the meat tenderness.

Calpain activity is influenced by calpastatin which is the endogenous specific inhibitor for protease -calpain. Increased post-mortem calpastatin activity is correlated with reduced meat tenderness. Variations in calpastatin gene change the activity of the gene and affect the tenderness of postmortem meat.

NGS technology is a technology that allows to analyze large amounts of DNA. It is therefore possible to perform sequence analysis of multiple genes or multiple exons simultaneously. We therefore used the NGS method to analyze the entire 31 exons of the CAST gene at the same time and on the same platform. Thus, we have ensured that many variations of the gene are included in the evaluation.

In this study, by performing whole gene sequencing instead of identified target mutations a large numberof variations was detected in the CAST gene. Positive or negative effects of the acquired polymorphisms on the CAST gene were evaluated.

Methods

Beef samples were obtained from two different cattle breeds, Brangus (n=52) and Simmental (n=52). Sequence analyses of the CAST gene were performed for each sample. A $1cm^3$ meat sample was taken for genetic analysis from each cattle for the two genetic groups and stored for DNA isolation. With appropriate adapter primers, 31 exons of the CAST gene and exon-intron binding areas were amplified. Genetic profiling uses "New Generation Sequencing" (NGS) method. The NGS outputs were analyzed in the Ensembl database. As a result of the analysis, 13 variations were determined. Unlike the Sanger sequencing and target polymorphism studies that were used in many previous surveys, this time a dynamic analysis was performed. With the NGS technology, more variations were determined and included in the study and also new variations were identified. Fisher's Exact test was utilized for statistical analysis of variations and group interactions.

Results

Distribution of variations acquired by analysis of Next Generation Sequencing (NGS) outputs.

A total of 13 variations (V1... → V13) were determined after analysis and bioinformatics evaluation with NGS (Table 1). V6 (exon 8 c.439C> G / p.L147LV) and V13 (intron 18 c.1335 + 6G> A) are variations that were not previously reported.

All variations that we have detected are the polymorphic attributes to MAF (minor allele frequencies) and only two of them (EXON 8 c.439c> G / p.l147v) and (INTRON 18 c.1535 + 6G> A) have not been previously reported and their pathogenicity is not clear and the "rs" number is not found.

Considering the variation distributions: the distribution frequencies of V1, V2, V5, V8, V10 and V13 variations were significantly different ($p < 0.05$) between Simmental and Brangus samples (Table 2).

Conclusion

In this study, the NGS method prevented the limitation of predetermined polymorphic variations in detecting the correlation between CAST gene and meat tenderness and provided us with the possibility to detect all possible polymorphisms. This genetic profiling data is highly important for cattle breeders and restaurant owners therefore theyare able to choose the type of meat or cattle according to the data of their genetic markers.

Notes

Variations		Brangus		Simmental		p-value
		n	Frequency	n	Frequency	
V1	No	25	48.1%	9	17.3%	0.001*
	Yes	27	51.9%	43	82.7%	
V2	No	35	67.3%	10	19.2%	0.000*
	Yes	17	32.7%	42	80.8%	
V3	No	48	92.3%	52	100.0%	0.059
	Yes	4	7.7%	0	0.0%	
V4	No	36	69.2%	29	55.8%	0.112
	Yes	16	30.8%	23	44.2%	
V5	No	45	86.5%	33	63.5%	0.006*
	Yes	7	13.5%	19	36.5%	
V6	No	50	96.2%	52	100.0%	0.248
	Yes	2	3.8%	0	0.0%	
V7	No	51	98.1%	47	90.4%	0.102
	Yes	1	1.9%	5	9.6%	
V8	No	52	100.0%	41	78.8%	0.000*
	Yes	0	0.0%	11	21.2%	
V9	No	52	100.0%	50	96.2%	0.248
	Yes	0	0.0%	2	3.8%	
V10	No	47	90.4%	52	100.0%	0.028*
	Yes	5	9.6%	0	0.0%	
V11	No	51	98.1%	52	100.0%	0.500
	Yes	1	1.9%	0	0.0%	
V12	No	51	98.1%	52	100.0%	0.500
	Yes	1	1.9%	0	0.0%	
V13	No	52	100.0%	38	73.1%	0.000*
	Yes	0	0.0%	14	26.9%	

TABLE 2. Frequencies and level of significance for variations detected in Brangus and Simmental beef

TABLE 1:Variations and their MAF (minor allele frequencies)values detected for the total beef cattle population

#	VARIATIONS	RS NUMBER*	MAF** (Ensembl)	MAF (Current Study)
1	EXON20 c.1526T>C/p.V509A	rs109384915	38%	42.78%
2	EXON 22 c.1632A>G/p.E544E (splice)	rs110712559	25%	36.53%
3	EXON 14 c.934A>G/p.N312D	rs723916435	23%	1.92%
4	EXON 9 c.583A>G/ p.T195A	rs210072660	44%	25%
5	EXON 9 c.616G>A/p.E206K	rs384020496	19,00%	12.5%
6	EXON 8 c.439C>G/ p.L147LV	undeclared	-	0.96%
7	INTRON 22 c.1714-3C>T	rs110711318	21%	2.88%
8	EXON 26 c.1985G>C/p.S662T	rs110914810	45%	8.65%
9	EXON 9 c.630G>AG/ p.K210KK	rs378682309	15%	1.92%
10	EXON13 c.895 G>A/ p.A299T	rs715323791	-	2.4%
11	EXON 20 c.1510C>T / P.P504S	rs1116977475	-	0.48%
12	INTRON 6 c.373-3C>T	rs433558933	-	0.48%
13	INTRON 18 c.1535+6G>A	undeclared	-	7.69%

*RS: Reference SNP cluster ID

Notes