Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

Haplotype association analysis of meat quality traits at the bovine PRKAG3 locus

E. Ciani, M. Roux, R. Ciampolini, M. Mazzanti, F. Cecchi, M. Tancredi, D. Cianci, H. Leveziel, S. Presciuttini & V. Amarger

To cite this article: E. Ciani, M. Roux, R. Ciampolini, M. Mazzanti, F. Cecchi, M. Tancredi, D. Cianci, H. Leveziel, S. Presciuttini & V. Amarger (2007) Haplotype association analysis of meat quality traits at the bovine PRKAG3 locus, Italian Journal of Animal Science, 6:sup1, 82-84, DOI: 10.4081/ijas.2007.1s.82

To link to this article: https://doi.org/10.4081/ijas.2007.1s.82

Haplotype association analysis of meat quality traits at the bovine PRKAG3 locus

E. Ciani[1], M. Roux[2], R. Ciampolini[3], E. Mazzanti[3], F. Cecchi[3], M. Tancredi[3], D. Cianci[1], H. Leveziel[2], S. Presciuttini[4], V. Amarger[5]

[1] Dipartimento di Fisiologia Generale ed Ambientale. Università di Bari, Italy
[2] Unité de Génétique Moléculaire Animale. Université de Limoges, France
[3] Dipartimento di Produzioni Animali. Università di Pisa, Italy
[4] Centro di Genetica Statistica. Università di Pisa, Italy
[5] UMR 1280 Physiologie des Adaptations Nutritionnelles. Centre INRA de Nantes, France

Corresponding author: Elena Ciani. Dipartimento di Fisiologia Generale ed Ambientale. Università di Bari. Via Amendola 165/A 70126 Bari, Italy - Tel. +39 080 5442413 - Fax: +39 080 5443388 - Email: elenaciani@biologia.uniba.it

ABSTRACT: The current study presents the results of a preliminary haplotype association analysis at the bovine *PRKAG3* locus with meat quality traits in the Chianina breed. No significant association was shown between haploid haplotypes (or diplotypes) and phenotypical traits after applying a Bonferroni correction for multiple comparison. Nonetheless, data from *Longissimus dorsi* muscle suggest the presence of a statistically non-significant trend toward an influence of the *PRKAG3* haploid haplotypes on meat colour (a*) and water holding capacity (M/T) traits, as confirmed also by diplotype-based association analysis. A less clear set of results was observed for the *Triceps brachii* and *Semitendinosus* muscles.

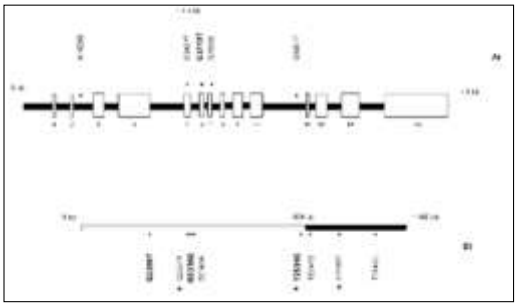
Key words: Meat quality, Chianina cattle breed, *PRKAG3*, Haplotype.

INTRODUCTION - The protein kinase adenosine monophosphate-activated γ3-subunit (*PRKAG3*) gene encodes the muscle specific isoform of the regulatory „ subunit of adenosine monophosphate activated protein kinase (*AMPK*), which plays a key role in regulating energy homeostasis in eukaryotes (Carling, 2004). Previous works indicated that substitutions in the first CBS domain of the *PRKAG3* gene affect glycogen content in muscle and influence meat quality traits in pigs, such as ultimate pH, meat colour, water-holding capacity, drip loss, tenderness and cooking loss (Milan *et al*., 2000; Ciobanu *et al*., 2001).
We report here a preliminary association analysis of haplotypes at the bovine *PRKAG3* locus with meat quality traits in the Chianina breed.

MATERIAL AND METHODS - A total of 97 not inbred young Chianina bulls (Cecchi *et al.*, 2001) raised in Tuscany were sampled. Animals had been slaughtered at approximately 19 months of age, after reaching a live weight of about 770 kg. According to the commercial aging periods for Chianina carcasses, the *T. brachii* (*Tb*) muscle was excised from the forequarters after about 10 days of aging, while the *L. dorsi* (*Ld*) and *Semitendinosus* (*St*) muscles were taken from the hindquarters after 19 days of aging (Preziuso and Russo, 2004). For each muscle, meat colour (lightness, L*, redness, a*, yellowness, b*, Croma, C* and Hue, H*, coordinates; Renerre, 1982), meat colour after 48h, water-holding capacity (drip loss, DL, cooking loss, CL and the M/T ratio; Hofmann *et al*., 1982), tenderness (Warner Bratzler shear force on raw, $WB_{(r)}$, and cooked, $WB_{(c)}$, meat) and chemical composition (dry matter, ether extract, crude protein and ash; A.O.A.C., 1990) were evaluated. Genomic DNA was isolated from whole blood following Jeanpierre (1987). In the whole, 13 single nucleotide polymorphisms (SNPs) were considered, spanning a region of 4.4 kb in the bovine *PRKAG3* gene (Fig. 1). A TaqMan® 5' allelic discrimination assay (Fig. 1-A; Applied Biosystems) and a sequencing approach (Fig. 1-B) using Big Dye Terminator chemistry on a ABI PRISM 3130 cycle sequencing were adopted to obtain genotypic data. Haplotypes were reconstructed from unphased genotypes using

the computer program PHASE v. 2.1 (Stephens *et al*., 2001). For each muscle, differences between haploid haplotype (or diplotype) groups for investigated phenotypes were assessed using one-way ANOVA, also considering the covariate effects of age at slaughtering and post-mortem aging time. A Bonferroni correction was applied to account for bias due to multiple tests by dividing the desired level of significance ($\alpha = 0.05$) by the total number of comparisons performed. The corrected false-positive rate was $\alpha = 0.0026$; all P values that fell below $\alpha = 0.026$ were considered experiment-wise significant. Analyses were performed using the software JMP v. 5.0 (2002).

Figure 1. Localisation of the thirteen considered SNP_s along the *PRKAG3* gene(*).



**SNPs encircled with dotted lines represent missense mutations. Stars indicate the three novel, unpublished, polymorphisms.*

Table 1. List of inferred haplotypes.

ID	Site variants (1)	N (2)	(p)*
H1	G T G G G T T C C C C G G	102	0,526
H2	A G G G G T G C T C C G G	64	0,330
H3	G G A G G C G T C C C G G	8	0,041
H4	G G G G A T G C C T C C T	7	0,036
H5	G G G G G T G C C C C G G	6	0,031
H6	G G A G G C G T T C C G G	4	0,021
H7	A G G G G T G C T C T G G	1	0,005
H8	A G G G G T G C C C C G G	1	0,005
H9	A G G C G T G C T C C G G	1	0,005

(1) Site variants are ordered by position along the chromosome.

(2) Number of analysed chromosomes.

** Haplotype frequencies.*

RESULTS AND CONCLUSIONS - The genotypes of all animals were submitted to the program PHASE v. 2.1 that implements a Bayesian approach in which the prior approximates the coalescent. The list of the different inferred haplotypes with their population frequencies are reported in Table 1. Almost all considered SNPs were in complete linkage disequilibrium to one each other (data not shown), allowing to infer the phase of the genotypes with high confidence. Out of the nine reconstructed haplotypes, only two showed frequency higher than 0.05 (H1, 52.6%, and H2, 33%). These two major haplotypes differed by four site variants (G1428A, T2260G, T2547G and C2643T); of these, only the SNP at position 2260 was located in a coding region (exon 4) and caused an amino acid substitution (A to S) at the residue 121 of the N-terminal part of the protein (Roux *et al*., 2006).

The growing need for product standardization and quality assurance in the beef chain strongly encourages to elucidate the genetic bases of meat quality traits at a molecular level. The role of the *PRKAG3* gene in influencing meat quality traits, such as pH, meat colour and water-holding capacity, has been clearly evidenced in pigs. In the current work we carried out a preliminary association analysis between *PRKAG3* haplotypes and beef quality traits in Chianina cattle. Results are summarised in Table 2. No significant association was shown between haploid haplotypes (or diplotypes) and phenotypical traits after applying a Bonferroni correction for multiple comparisons. Unfortunately, this method of accounting for the inflation of the alpha level due to multiple tests, though being simple and easy to compute, is one of the most stringent multiple testing correction methods; it therefore severely reduces the power to detect potential significant associations. Considering a trait-wise critical value of $P<0.05$, meat colour (a*) and water holding capacity (M/T) traits were significantly associated with *PRKAG3* haploid haplotypes in the *L. dorsi* muscle (also confirmed by diplotype-based association analysis), while a less clear set of results was observed for the *T. brachii* and *Semitendinosus* muscles. Further studies are, therefore, required to delineate the role of the protein kinase on meat quality attributes, and combination of genomic and proteomic approaches should be advisable.

Table 2. Results of the association analysis for the three analysed muscles.

Method	*T. brachii*		*L. dorsi*		*Semitendosus*	
	Trait	*P value*	*Trait*	*P value*	*Trait*	*P value*
Haplotype-based association analysis	Proteins	0.0059	a*	0.0443	Ashes	0.0273
	-	-	M/T	0.0470	L*	0.0382
	-	-	-	-	L*48	0.0347
	Trait	*P value*	*Trait*	*P value*	*Trait*	*P value*
Diplotype-based association analysis	-	-	a*	0.0354	EE	0.0253
	-	-	C*	0.0406	-	-
	-	-	M/T	0.0366	-	-

The Authors want to thank Prof. Giovanna Preziuso and Dr. Claudia Russo (Dipartimento di Produzioni Animali, Università di Pisa, Italia) for providing meat instrumental measurements, Dr. Ahmed Ouali (QuaPA, BPM, INRA de Clermont Ferrand – Theix, France) for helpful discussion and the I.Z.S.U.M. (Director Dr. Angelo Foglini), which contributed with financial support to this work.

REFERENCES - A.O.A.C. 1990. Official methods of analysis. 15th ed. AOAC, Arlington, USA. **Carling,** D., 2004. The AMP-activated protein kinase cascade—a unifying system for energy control. Trends Biochem. Sci., 29:18–24. **Cecchi**, F., Ciampolini, R., Leotta, R., Mei, D., Polizzi, E., Cianci, D., 2001. La stima della variabilita' genetica della razza chianina attraverso i dati genealogici e molecolari: il confronto tra parentele e rassomiglianza genetica. Annali della Facoltà di Medicina Veterinaria, Pisa LIV:175-186. **Ciobanu,** D., Bastiaansen, J., Malek, M., Helm, J., Woollard, J., Plastow, G., Rothschild, M., 2001. Evidence for new alleles in the protein kinase adenosine monophosphate-activated gamma(3)-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics,159(3):1151-62. **Hofmann,** K., Hamm, R. and Bluchel, E., 1982. New information on the determination of water binding in meat by the filter paper press method. Fleischwirtsch 62:87-94. **Jeanpierre,** M., 1987. A rapid method for the purification of DNA from blood. Nucleic Acids Res., 15: 9611. **JMP** v. 5.0. 2002. SAS Institute, Cary, N.C. **Milan,** D., Jeon J.-T., Looft, C., Amarger, V., Robic, A., *et al*. 2000. A mutation in *PRKAG3* associated with excess glycogen content in pig skeletal muscle. Science, 288:1248-1251. **Preziuso,** G.,. and Russo, C., 2004. Meat quality traits of longissimus thoracis, semitendinosus and triceps brachii muscles from Chianina beef cattle slaughtered at two different ages. Ital. J. Anim. Sci., 3:267-273. **Renerre,** M., 1982. La couleur de la viande et sa mesure. Bulletin Technique, C. R. Z. V. Theix I.N.R.A, 47: 47-54. **Roux,** M., Nizou, A., Forestier, L., Ouali, A., Leveziel, H., Amarger, V., 2006. Characterization of the bovine *PRKAG3* gene: structure, polymorphism, and alternative transcripts. Mammalian Genome, 17: 83-92. **Stephens,** M., Smith, N.J., Donnelly, P., 2001. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68:978-989.