Research in Veterinary Science 90 (2011) 245–252

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

History and selection imprinting on genetic relationships among bovine breeds analyzed trough five genes related with marbling

M.V. Ripoli *, A. Rogberg-Muñoz, J.P. Lirón, E. Francisco, E.E. Villegas-Castagnasso, P. Peral-Garcia, G. Giovambattista

Instituto de Genética Veterinaria (IGEVET) CCT La Plata – Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata-CONICET, Argentina

ARTICLE INFO

Article history:
Received 3 November 2009
Accepted 14 June 2010

Keywords:
Bovine
Single nucleotide polymorphism
Genetic diversity
Marbling
Phylogeography
Selection

ABSTRACT

Many candidate genes have been suggested as responsible for marbling in beef cattle, for instance diacylglycerol O-acyltransferase 1, thyroglobulin, growth hormone, leptin and stearoyl CoA desaturase. The objective of the present work was to evaluate the polymorphisms of five SNPs of these candidate genes in 389 animals of 18 *Bos Taurus* and *Bos indicus* breeds. The obtained results were compared with ones previously obtained with STRs and loci related to milk production in these populations. Moreover we analyzed whether the phylogenies reconstructed using SNPs associated with marbling resulted in the known tree topology. The tree constructed with UPGMA, using genetic distance D_A, exhibit a topology partially consistent with the historical origin of breeds. The result observed in the Correspondence Analysis coincided with the topology of the UPGMA tree. This work allowed us to evaluate the five SNPs genetic diversity and to demonstrate that the grouping of the breeds may be the result of its history, selection process, or both at once.

1. Introduction

Marbling is an important trait for meat quality because confers juiciness, flavor and tenderness to beef hence it contributes directly to the price of beef on international markets. Many candidate genes have been suggested as responsible for marbling in beef cattle, such as diacylglycerol O-acyltransferase1 (DGAT1), thyroglobulin (TG), growth hormone (GH), leptin (LEP) and stearoyl-coenzyme A desaturase (SCD) (Barendse et al., 1999, 2001, 2004, 2006; Buchanan et al., 2002; Thaller et al., 2003; Nkrumah et al., 2004a,b; Taniguchi et al., 2004; Sorensen et al., 2006; Oka et al., 2002; Tatsuda et al., 2008).

The DGAT1 is a microsomal enzyme that catalyzes the final step of triglyceride synthesis. The DGAT1 gene has been mapped to bovine chromosome 14. A lysine/alanine (K232A) substitution on the protein encoded by the bovine DGAT1 gene has been shown to be associated with milk fat content in different breeds such as Holstein–Friesian, Fleckvieh and Jersey (Grisart et al., 2002; Spelman et al., 2002; Winter et al., 2002), and with fat deposition in beef cattle. Thaller et al. (2003) showed that the lysine allele of DGAT1 has also a positive effect on intramuscular fat content in the Charolais and Holstein breeds. Moreover, Sorensen et al. (2006) reported that the DGAT1 activity in *longissimus dorsi* muscle in individuals with K/K genotype was about five fold greater than for either the K/A or A/A genotypes in Holstein and Charolais bulls. In contrast, Moore et al. (2003) detected no association of the SNP in the DGAT1 gene (K232A mutation) with fat thickness in a commercial line of *Bos taurus*. In addition, Casas et al. (2005) reported no significant associations of DGAT1 alleles with carcass composition and meat quality traits in *Bos indicus*.

The T3 and T4 thyroid hormones have an important role in the metabolic regulation, and among other functions, they affect the lipid metabolism. TG is the precursor of T3 and T4 in the thyroid gland and its gene has been mapped to bovine chromosome 14. By this reason, the TG gene has been considered as a candidate gene to explain differences in marbling. Barendse et al. (2001) reported the C to T transition in the thyroglobulin 5′ leader sequence to be highly associated with intramuscular fat deposition in long-fed cattle. This transition defines the '2' (C) and '3' (T) alleles. Barendse et al. (1999, 2004) found that the TG '3' allele was more frequent in animals with higher marbling scores. However, this marker appears to be useful in Wagyu cattle specifically. In other beef cattle breeds this marker has not proved to be a good predictor of marbling (Rincker et al., 2006; Barendse et al., 2004; Casas et al., 2005, 2007).

As was mentioned above, DGAT1 and TG genes have been mapped to the centromeric region of chromosome 14. The presence of a quantitative trait locus (QTL) in the centromeric end of chromosome 14 associated with production traits in cattle has

* Corresponding author. Address: IGEVET – CCT La Plata – FCV – CONICET, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata B1900AVW, CC 296, Argentina. Tel./fax: +54 221 4211799.
E-mail address: mvripoli@fcv.unlp.edu.ar (M.V. Ripoli).

0034-5288/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2010.06.019

been supported by many studies (Coppieters et al., 1998; Heyen et al., 1999; Riquet et al., 1999; Looft et al., 2001; Boichard et al., 2003).

GH is a polypeptide hormone secreted by the anterior pituitary gland and it plays a major role in tissue growth, fat metabolism and homeorhesis (Shingu et al., 2004; Beauchemin et al., 2006; Thomas et al., 2007). The GH gene is the regulator of the animal growth and metabolism and it has been mapped to bovine chromosome 19. Different polymorphisms have been identified in the bovine GH gene (Lucy et al., 1991; Zhang et al., 1993; Kirkpatrick et al., 1993). Most of these polymorphisms have been associated with differences in carcass composition, marbling and milk production (Lee et al., 1996; Yao et al., 1996; Lechniak et al., 2002; Di Stasio et al., 2005; Curi et al., 2005; Barendse et al., 2006; Thomas et al., 2007). In the present report the analyzed polymorphism was GH6.1, also known as AluI RFLP (Yao et al., 1996). It is caused by a C to G nucleotide change in the fifth exon of the gene, which gives rise to two alleles that are responsible for alternative forms of bovine GH with a Leucine or Valine amino acid residue at position 127.

LEP is a protein hormone product of the obese gene synthesized and secreted predominantly by white adipocytes (Zhang et al., 1994; Ji et al., 1998). The role of LEP as a lipostatic signal that regulates whole-body energy metabolism makes it one of the best physiological markers of body weight, food intake, energy expenditure (Houseknecht et al., 1998; Woods et al., 1998), reproduction (Cunningham et al., 1999; Garcia et al., 2002), and certain immune system functions (Lord et al., 1998). LEP gene has been mapped to bovine chromosome 4 (Stone et al., 1996). Polymorphisms in the coding regions of the leptin gene in cattle have been associated with serum leptin concentration (Liefers et al., 2003), feed intake (Liefers et al., 2002; Oprzadek et al., 2003), milk yield (Liefers et al., 2002; Buchanan et al., 2003), body fatness (Buchanan et al., 2002; Nkrumah et al., 2004a,b) and with marbling scores (http://ca.igenity.com/igenity_beef1.html). We analyzed the polymorphism situated in exon 3 of the leptin gene (Liefers et al., 2002) which causes an amino acid change from Alanine to Valine amino acid residue at position 59.

SCD is the enzyme responsible for the conversion of saturated fatty acids to Δ9-monounsaturated fatty acids. Inhibition of desaturase activity leads to an accumulation of stearic acid in bovine adipose tissue, which can cause a substantial increase in fat hardness (Smith et al., 1998; Yang et al., 1999). The fatty acid composition of bovine fat has an impact on the visual manifestation of marbling during processing, the softness of the fat, and the flavour of the meat on the consumers plate (Melton et al., 1982; Smith et al., 1998). Due to its important role in fatty acid oxidation, SCD is a candidate for genetic variation in fatty acid composition. Taniguchi et al. (2004) reported in Japanese Black cattle an aminoacid substitution on the SCD gene that may change the enzymes's catalytic activity. This SNP, observed in the ORF (position 878) of SCD gene, causes an amino acid replacement from Valine (V) to Alanine (A).

On the other hand, several studies have reported geographical clines in polymorphism on genes related with production traits, such as α_{S1}-cas, κ-cas, GH, serum albumin, several microsatellites and Y-chromosome polymorphisms. These gradients have been shown to be related to different causes, such as domestication centre, population origin, migration route, gene introgression and/or adaptive effects of a particular allele (Baker and Manwell, 1980; Medjugorac et al., 1994; MacHugh et al., 1994, 1997; Lirón et al., 2002; Beja-Pereira et al., 2002, 2003).

The objective of the present work was to evaluate the polymorphisms of five SNPs of candidate genes related with marbling: DGAT1, TG, LEP, GH and SCD in 389 animals of 18 *B. Taurus* (European, Asian and Creole) and *B. indicus* breeds, in order to evaluate the genetic diversity within and among studied populations and the phylogenetic relationship of the analyzed breeds. The results obtained from the analysis of the SNPs were compared with ones previously obtained with five loci related to milk production (Lirón et al., 2002) and nine microsatellites (Lirón et al., 2006) in the same populations. Moreover we wanted to check whether the phylogenies reconstructed using SNPs associated with marbling resulted in the known tree topology.

2. Materials and methods

2.1. Sample collection

Blood samples were collected from 389 animals belonging to 18 *B. Taurus*, *B. indicus* (Brahman and Nelore) and synthetic Brangus breeds (Table 1). The *B. Taurus* breeds were grouped according to their geographical origin in European (Hereford, Aberdeen Angus, Galloway, Holstein, Jersey, Charolais and Retinta), Asian (Wagyu) and American Creole breeds (Argentine Creole, Patagonian Creole, Saavedreño Creole, Chaqueño Boliviano, Chusco, Valle Grande Creole, Yacumeño Creole).

2.2. DNA extraction

Total DNA was extracted from blood samples using the DNAzol® reagent (Invitrogen, Carlsbad, CA, USA) following manufacturer instructions.

2.3. SNPs genotyping

The five SNPs of candidate genes related with marbling were analyzed by PCR-RFLP or PCR-SSCP as detailed in Table 2.

Table 1
Summary of cattle breeds sampled.

Breed	Acronyms	*N*	Breed origin	Sample origin
Hereford	HE	21	England	Argentine
Aberdeen Angus	AA	59	Scotland	Argentine–Uruguay
Galloway	G	10	Scotland	Argentine
Holstein	HO	20	Netherlands	Argentine
Jersey	J	10	Island of Jersey	Argentine
Charolais	CH	14	France	Uruguay
Retinta	T	26	Spain	Spain
Argentine Creole	AM	20	Argentine	Argentine
Patagonian Creole	CA	20	Argentine	Argentine
Saavedreño Creole	SAA	20	Bolivia	Bolivia
Chaqueño Boliviano	ES	20	Bolivia	Bolivia
Chusco	PA	7	Bolivia	Bolivia
Valle Grande Creole	V	20	Bolivia	Bolivia
Yacumeño Creole	Y	35	Bolivia	Bolivia
Brangus	BR	12	EE.UU	Argentine
Brahman	BZ	20	EE.UU	Bolivia
Nelore	NE	33	Brasil	Argentine–Bolivia
Wagyu	W	22	Japan	Uruguay

Table 2
Genotyped method, analyzed mutation and reference for each studied SNP.

SNP	Method	Analyzed mutation	Author
DGAT1	PCR-SSCP	K232A (eighth exon)	Ripoli et al. (2006)
TG	PCR-RFLP	C → T (5́ leader sequence)*	Barendse et al. (2001)
LEP	PCR-RFLP	A59 V (third exon)	Liefers et al. (2002)
GH	PCR-RFLP	L217 V (fifth exon)	Yao et al. (1996)
SCD	PCR-RFLP	Val → Ala (878 ORF position)	Taniguchi et al. (2004)

* This transition defines the '2' (C) and '3' (T) alleles.