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A note on the growth hormone (*GH1-Alu*I) polymorphism in Podolian cattle in Southern Italy[1,*]

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Individual blood samples were collected from 132 unrelated Podolian cattle belonging to 13 farms located in the inland territories of Southern Italy. Genomic DNA samples were genotyped for the *GH1-Alu*I polymorphism by Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP). The mutation analysed causes the amino acid substitution leucine→valine at position 127 of the GH polypeptide. Allele frequencies for *GH1 A* and *GH1 B* were 0.85 and 0.15, respectively. Hardy-Weinberg equilibrium was found in the analysed animals. Due to the rather low frequency of *GH1 B* in the breed, a larger sample including a higher number of animals carrying *GH1 B* is necessary to assess possible association between *GH1-Alu*I genotypes and beef traits in the Podolian cattle.

KEY WORDS: **cattle / gene polymorphism / growth hormone / Podolian breed / PCR-RFLP**

Growth hormone (GH) plays a central role in the regulation of growth and metabolism in animals [Carnicella *et al*. 2003, a review]. The bovine *GH1* is a single copy gene that spans 1800 bp and consists of five exons, associated with chromosome region 19q26-qter [Hediger *et al*. 1990]. Several mutations have beed identified in the bovine growth hormone gene [Chikuni *et al*. 1991, Zhang *et al*. 1993, Unanian *et al*. 1994, Lagziel and Soller 1999]. The most extensively studied polymorphism was the exon 5 C→G transversion at *GH1* nucleotide position 2141 of the *GH1* reference sequence [Gordon *et al*. 1983] – GenBank accession number M57764 – causing the amino acid substitution leucine→valine at position 127 of the GH polypeptide [Wallies and Dav-

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ies 1976, Chikuni *et al*. 1991]. This single nucleotide polymorphism (SNP) resulting in two alleles – *GH1 A* and *GH1 B* – was found in all the taurine breeds analysed, while monomorphism for *GH1 A* occurred in zebu (*Bos indicus*) breeds (Tab. 1). The two *GH1* variants are often reported as *GH1L* and *GH1V*, according to the amino acid substitution involved [Schlee *et al*. 1994a, Sabour *et al*. 1997].

The Podolian breed of cattle derives from *Bos primigenius podolicus* (http://www.anabic.it/servizio_tecnico/St56uk.htm), and has spread throughout an area that mainly covers the inland territories of Southern Italy. The breed numbers about 100,000 head, 25% of which are listed in the Italian Herd Book of ANABIC (National Association of Italian Beef Cattle Breeders), responsible for the development and selection of the breed. One of the outstanding characteristics of Podolian cattle is their ability to adapt to exceptionally difficult environment, as well as extraordinary capacity to utilize feed resources that would not otherwise be used. The Podolian cattle have long been used mainly as working and only secondarily as beef or dairy animals. In fact, their milk is ideal for producing the famous "caciocavallo" cheese. Subsequently, with the rise and spread of mechanization in agriculture, the selection of the breed was focused on beef and, to a lesser extent, on dairy production, particularly in certain areas. The present selection aims at obtaining animals with a marked ability to be raised in open-pasture or semi-open-pasture (ranch-like) extensive systems, particularly in difficult environments with poor-quality and (or) varying forage resources, yielding high-quality beef. Excellent maternal behaviour and long life-span are other important selection goals.

The aim of the present study was to analyse *GH1-Alu*I polymorphism in Podolian cattle and to compare it with the gene frequencies found in different bovine breeds by different authors. This first molecular approach intends to be an introductory step for genetic characterization of this indigenous cattle and is preliminary to a wider studies upon the possible associations between *GH1-Alu*I and beef traits in the breed.

Material and methods

A total of 132 individual blood samples were randomly withdrawn from Podolian cattle (both sexes) belonging to 13 farms located in the inland territories of Basilicata and Puglia (Apulian) regions of Italy. Blood was obtained from jugular vein on K_2EDTA by authorized veterinarian and stored at -25°C for few days. Genomic DNA was extracted from blood by a rapid method using GFX genomic kit (AMERSHAM, Germany).

Genotyping for *GH1-Alu*I polymorphism was performed by Polymerase Chain Reaction – Rectriction Fragment Length Polymorphism (PCR-RFLP) according to Reis *et al*. [2001], using an Eppendorf Personal thermalcycler, HotMasterMix (EPPENDORF, Italy) with 50 ng of bovine genomic DNA, and 16 pmol of each primer. The amplified 211 bp-long PCR product was digested with *Alu*I restriction endonuclease (SIGMA-ALDRICH, USA) and separated in 2% agarose gel.

Results and discussion

In homozygous animals either a unique band (211 bp, *GH1 BB* genotype), or two-band (159 and 52 bp, *GH1 AA* genotype) patterns were observed (Fig. 1). Heterozygous animals showed a three-band (211, 159 and 52 bp) pattern. Gene frequencies of alleles *GH1 A* and *GH1 B* were 0.85 and 0.15, respectively (Tab. 1), confirming previous report on a small number of *GH1 B* animals by Dario *et al*. [2004].

Moreover, Table 1 presents a comparison of *GH1 A* and *GH1 B* allele frequencies in Podolian cattle (present study) with those gathered for other cattle breeds. Frequencies are arranged in ascending order for *GH1 A* which is the predominant allele in all breeds, with frequencies ranging from 0.56 to 1. A great inter- and intra-breed variation was found in frequencies of growth hormone polymorphic variants in cattle in the United States [Lucy *et al*. 1993] and in Canada [Sabour *et al.* 1997]. In the lower part of Table 1, four breeds are presented, all being monomorphic for *GH1 A*. Three of them (Nelore, Gyr, Guzerá) are zebu (*Bos indicus*) breeds. Moreover, Santa Gertrudis and Canchim are *Bos indicus* × *Bos taurus* crosses. Due to the rather low frequency of *GH1 B*, Podolian cattle entered the second half of the table, just before some dairy breeds (Brown, Holstein, Guernsey), the two *Bos indicus* × *Bos taurus* crosses, and the *Bos indicus* group.

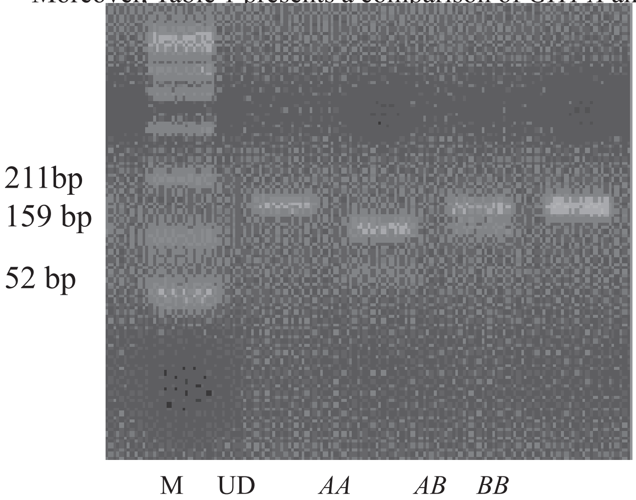


Fig. 1. Electrophoretic patterns of *AA*, *AB* and *BB* genotypes. (M= molecular marker; UD= undigested).

The animals considered in this study remained in a Hardy-Weinberg equilibrium (Tab. 2), the overall chi-square value amounting to 0.486 (0.548 for Basilicata and 0.035 for Apulian regions) being associated to 1 degree of freedom and to a probability higher than 0.485.

As nowadays the main purpose of keeping Podolian cattle is beef production, the

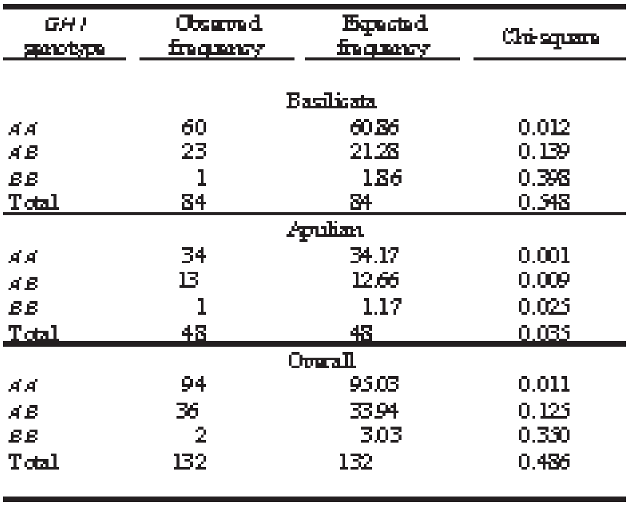
Table 1. Frequencies of the alleles *GH1 A* and *GH1 B* in Podolian breed and in different cattle breeds analysed by other authors. Allele frequencies are shown in increasing order for *GH1 A*

Breed	Allele		Reference
	A	*B*	
Jersey	0.56	0.44	Lucy *et al.*, 1993
Slovak Simmental	0.56	0.44	Chrenek *et al.*, 1991
Limousine	0.67	0.33	Dybus *et al.*, 2003
Bavarian Simmental	0.68	0.32	Schlee *et al.*, 1994a
Ayrshire	0.71	0.29	Sabour *et al.*, 1997
Simmental	0.71	0.29	Schlee *et al.*, 1994b
Charolais	0.72	0.28	Kemenes *et al.*, 1999
Piemontese	0.72	0.28	Di Stasio *et al.*, 2002
Jersey	0.76	0.24	Sabour *et al.*, 1997
Angus	0.77	0.23	Regitano *et al.*, 2000
Hereford	0.78	0.22	Chikuni *et al.*, 1991
Ayrshire	0.79	0.21	Lucy *et al.*, 1993
Angus	0.80	0.20	Chikuni *et al.*, 1991
Caracu	0.80	0.20	Kemenes *et al.*, 1999
German Black and White	0.80	0.20	Schlee *et al.*, 1994b
Podolian	**0.85**	**0.15**	**this study**
Bavarian and Tyrolean	0.90	0.10	Schlee *et al.*, 1994b
Brown			Schlee *et al.*, 1994b
Canchim	0.90	0.10	Kemenes *et al.*, 1999
Holstein	0.91	0.09	Sabour *et al.*, 1997
Guernsey	0.92	0.08	Lucy *et al.*, 1993
Holstein	0.92	0.08	Vukasinovic *et al.*, 1999
Holstein	0.93	0.07	Lucy *et al.*, 1993
Santa Gertrudis	0.97	0.03	Kemenes *et al.*, 1999
Brown Swiss	1.00	0.00	Lucy *et al.*, 1993
Guzerá	1.00	0.00	Kemenes *et al.*, 1999
Gyr	1.00	0.00	Kemenes *et al.*, 1999
Nelore	1.00	0.00	Kemenes *et al.*, 1999

effects of growth hormone genotypes on growth traits are of great interest for their breeders. In the majority of studies on relationship between *GH1* genotype and meat traits in cattle, animals with genotype *BB* demonstrated lower growth rate than individuals with the two other genotypes. The *BB* individuals showed lower body weight and daily gain [Chrenek *et al*. 1998; Sirotkin *et al*. 2000], lower meat deposition [Oprzadek *et al*. 1999] and lower weight of meat in the carcass [Grochowska *et al.* 2001]. Recently, however, Zwierzchowski *et al*. [2001] found that *BB* beef bulls had higher daily weight gain than the bulls of other genotypes, and Di Stasio *et al.* [2002] reported no association between *GH1* polymorphism and meat production traits in Piedmontese cattle.

The present report intends to be a first step in genetic charcterization of Podolian cattle reared in Italy. In the breed, the frequency of *Alu*I (-) allele, corresponding to the

Table 2 Observed and expected genotype *GH1-Alu*I frequencies and chi-square values under the hypothesis of Hardy-Weinberg equilibrium in two Podolian breed populations belonging to Basilicata and Apulian region



GH1 genotype	Observed frequency	Expected frequency	Chi-square
		Basilicata	
AA	60	60.86	0.012
AB	23	21.28	0.139
BB	1	1.86	0.398
Total	84	84	0.548
		Apulian	
AA	34	34.17	0.001
AB	13	12.66	0.009
BB	1	1.17	0.025
Total	48	48	0.035
		Overall	
AA	94	95.03	0.011
AB	36	33.94	0.125
BB	2	3.03	0.330
Total	132	132	0.486

B variant which is associated with Val_{127} in the polipeptide sequence, was rather low. Nevertheless, further investigation is necessary to perform a robust statistical analysis aiming at establishing or denying the existence of association between *GH1-Alu*I genotypes and meat traits in the Podolian breed of cattle.

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Polimorfizm genu hormonu wzrostu (*GH1-Alu*I) włoskiego bydła podolskiego ("*Podolian cattle*")

Streszczenie

Krew pobrano od niespokrewnionego ze sobą bydła podolskiego (n=132) utrzymywanego w 13 fermach na terenie południowych Włoch, w rejonach Basilicata i Apulia. Bydło zwane tu podolskim reprezentowało do niedawna typ użytkowy roboczy, a obecnie prowadzone są prace nad przekształceniem go w typ dwukierunkowy, z przewagą mięsności. Próbki genomowego DNA genotypowano pod kątem widzenia polimorfizmu genu *GH1-Alu*I, posługując się metodą PCR-RFLP. Analizowana mutacja polega na zastąpieniu leucyny waliną w pozycji 127 polipeptydu GH. Frekwencje alleli *GH1 A* i *GH1 B* wyniosły odpowiednio 0,85 i 0,15. Badany materiał pozostawał w równowadze genetycznej zgodnie z prawem Hardy-Weinberga. Autorzy wnioskują, że z racji niskiej częstości występowania *GH1 B*, do wykrycia ewentualnego związku między polimorfizmem genu *GH1-Alu*I a cechami mięsności bydła podolskiego potrzebne jest zbadanie większej populacji zwierząt, która zawierałaby więcej nosicieli *GH1 B*.