



Polymorphism of Growth Hormone GH1-AluI in Jersey Cows and Its Effect on Milk Yield and Composition

C. Dario*, D. Carnicella, E. Ciotola¹, V. Peretti² and G. Bufano

Department of Health and Welfare of Animals, University of Studies of Bari- Italy

ABSTRACT : To analyze genetic effects on milk production traits, 164 unrelated Italian Jersey cows reared in 15 farms located in Southern Italy were characterized at the growth hormone locus. Using a Polymerase Chain Reaction-Restriction Fragment Length Polymorphism protocol all three possible genotypes for the Leucine/Valine polymorphism were identified even if in the considered population the number of homozygous animals (Leucine/Leucine = 36; Valine/Valine = 28) was smaller than Leucine/Valine (n = 100) cows; the Hardy-Weinberg equilibrium was verified. Statistical analysis showed that daily milk yield in the Leucine/Leucine genotype was higher ($p < 0.01$) than in Leucine/Valine (22.76 kg vs. 16.96 kg); cows with Leucine/Valine genotype differed significantly from Leucine/Leucine and Valine/Valine genotypes particularly in fat (4.95% vs. 4.13% and 4.82%, respectively) and protein content (4.00% vs. 3.47% and 3.79%, respectively). No significant effect on lactation length was observed. (**Key Words :** Growth Hormone, Polymorphism, Jersey, Milk Traits)

INTRODUCTION

Variations at DNA level contribute to the genetic characterization of livestock populations and sometimes a variation in the exonic region of a gene may lead to changes in amino acids which alter the expressed protein. Moreover, although intronic variation cannot change the amino acid sequence of the protein, it may play a significant role in gene splicing or binding of regulatory proteins during transcription. In livestock, such variations in DNA may also be associated with, or linked to, economic traits, which are governed by many genes each having a small effect (Gelderman, 1997). When a gene, like growth hormone, is usually involved in the biology of a trait, it can be considered as a candidate gene for marker identification. Particularly interesting in cattle is the Leu/Val polymorphism, a single-base polymorphism in the fifth exon. This Leu/Val polymorphism produces two variants of GH that differ in the presence of either leucine (Leu) or valine (Val) at amino acid residue 127 (Lucy et al., 1991).

Since this nucleotide sequence polymorphism may also change the biological properties of the growth hormone, it has been studied as a potential genetic marker using a candidate gene approach for milk yield (Van der Werf et al., 1996). Indeed, Eppard et al. (1992) have shown that injections of recombinant GH with the valine residue improved milk production to a greater extent than the same injections with the leucine residue, suggesting that the leucine residue can affect the properties of GH since the mutation is functional.

The allele frequency differs between breeds, in fact a greater inter- and intra-breed variation was found in dairy cattle (Table 1).

The aim of this study was to analyse bGH gene polymorphism in the Italian Jersey breed and to verify its effect on milk production traits.

MATERIALS AND METHODS

Animals

Although the Italian Jersey cow is still a little-known cattle breed despite its good characteristics, it is finally winning the national market and the number of its breeders seems to be increasing (www.AIA.it). The main characteristics of Jersey milk are the elevated caloric power and the good quality, due to the fat and protein content and

* Corresponding Author: C. Dario. Tel: +39-080-5443918, Fax: +39-080-5443925, E-mail: c.dario@veterinaria.uniba.it

¹ University of Catanzaro "Magna Graecia", Catanzaro -Italy.

² Department of Animal Science and Food Inspection, University Federico II, Naples - Italy.

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Table 1. Frequencies of the alleles GH1 A and GH1 B in different cattle breeds (Allele frequencies are shown in increasing order for GH1*A)

Breed	Alleles		References
	A	B	
Jersey	0.56	0.44	Lucy et al. (1993)
Ayrshire	0.71	0.29	Sabour et al. (1997)
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)
Ayrshire	0.79	0.21	Lucy et al. (1993)
German Black and White	0.80	0.20	Schlee et al. (1994b)
Podolian	0.85	0.15	Dario et al. (2004)
Bavarian and Tyrolean Brown	0.90	0.10	Schlee et al. (1994b)
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)
Brown Swiss	1.00	0.00	Lucy et al. (1993)

to the superior calcium concentration, which is its strongest point and one of the factors that led to the breed's geographical diffusion, making it the second milk breed in the world. Especially destined to making of the best-known Italian cheeses (Parmigiano-Reggiano, Grana-Padano, Asiago, Taleggio), the milk of the Jersey has enriched the strong local tradition of typical dairy products thanks to its taste and quality.

But the Jersey breed has other features also such as the extreme adaptability to all breeding types and, thanks to its great sturdiness, it is less subject to the pathologies resulting from intensive breeding and therefore easier to manage.

Genotyping for GH

Blood samples for DNA genotyping were obtained from 164 Jersey cows, collected in K-EDTA tubes and stored at -25°C. After genomic DNA isolation the cows were genotyped for the Leu/Val polymorphism in the GH gene. Genotypes were identified with the PCR-RFLP protocol described by Reis et al. (2001). The primer sequences were as follows: GH5 (*forward*): 5'- GCT GCT CCT GAG GGC CCT TC- 3'; GH5 (*reverse*): 5'- CAT GAC CCT CAG GTA CGT CTC CG- 3'.

The 281-bp bGH gene fragment, covering a part of the fourth intron and part of the adjacent fifth exon, was amplified using thirty amplification cycles under the following conditions: 94°C/30 s 60°C/L min and 72°C/30 s. The amplified 281 bp product was digested with *AluI* restriction endonuclease (Sigma; 3 h, 10 units/20 µl, 37°C) and analysed on a 2% agarose gel in TBE buffer.

Milk composition

Individual milk lactation data were collected from 164 primiparous unrelated cows, bred in 15 farms located in

Southern Italy and calved from October 2004 to June 2005. The cows were milked twice a day and individual samples were taken from morning and evening milking once a month during the whole lactation until they were dried off. Daily milk yield, considered as individual yield per lactation divided by total number of days in lactation, was calculated; milk composition was assessed in samples obtained for each animal from monthly milk records. In particular, milk fat (% and kg/d) and protein (% and kg/d) were determined by infrared spectroscopy on a MilkoScan 255. The lactation length was considered to be from calving to dryness.

Statistical analysis

The GH allele frequencies were calculated by simple allele counting according to the Hardy-Weinberg equilibrium (Falconer and Mackay, 1996); the possible deviations of genotype frequencies from expectation were tested by chi-square.

Effects of polymorphic variants of the growth hormone gene on milk performance traits were analysed using the GLM procedure of SAS (Sas/Stat User's guide, 1999).

The following model was used:

$$Y_{ijkl} = \mu + G_i + F_j + YS_k + e_{ijkl}$$

where: Y_{ijkl} is the analysed trait of the cow (milk, fat, protein, lactation length); μ is the overall mean; G_i is the fixed effect of the i^{th} genotype (1, ..., 3); F_j is the fixed effect of the j^{th} farm (1, ..., 15); YS_k is the fixed effect of the k^{th} season of calving (1, ..., 3); and e_{ijkl} = random error.

Dominance effects were estimated as the deviation of mean values of the studied traits in heterozygotes from the mean of homozygotes, using the least square means. Additive effect was calculated as half of the difference

Table 2. Observed and expected numbers and percentages (in brackets) of GH genotypes detected by AluI RFLP analysis and allele frequencies in the sample of Italian Jersey cows

Number	GH genotype			Allele frequency	
	<i>Leu/Leu</i>	<i>Leu/Val</i>	<i>Val/Val</i>	<i>Leu</i>	<i>Val</i>
Observed	36 (21.95%)	100 (60.98%)	28 (17.07%)	0.52	0.48
Expected	45.1 (27.5%)	81.8 (49.88%)	37.1 (22.62%)		
$\chi^2 = 8.11$	$p \leq 0.005$				

Table 3. Least squares means and their standard error for daily milk fat and protein yield (percentage and yield) and lactation length for each GH genotype

Traits	Genotypes		
	<i>Leu/Leu</i>	<i>Leu/Val</i>	<i>Val/Val</i>
Milk (kg/d)	22.76±0.41 ^{Aa}	16.96±0.23 ^B	18.47±0.57 ^b
Fat			
(%)	4.63±0.07 ^a	4.96±0.04 ^b	4.84±0.11
(kg/d)	0.94±0.02 ^a	0.84±0.01 ^b	0.89±0.03
Protein			
(%)	3.84±0.03 ^a	4.00±0.02 ^b	3.81±0.04 ^a
(kg/d)	0.79±0.01 ^A	0.68±0.01 ^B	0.70±0.02 ^B
Length (d)	321.33±7.08	318.36±4.45	315.00±11.12

Within rows, the means with different superscripts significantly differ (^{A,B} $p < 0.01$; ^{a,b} $p < 0.05$).

between the two homozygotes. The significance of these factors was detected by the method of least squares difference.

RESULTS AND DISCUSSION

Gene frequency

Three patterns (genotypes) were produced as the result of AluI restriction. Two (LL), one (VV) and three (LV) band patterns could be distinguished on the gel, which are the products of two alleles (L and V).

The expected allele frequencies were calculated according to the Hardy-Weinberg equilibrium:

$$pL = \frac{2 \text{ GH LL} + \text{GH LV}}{2N} \quad \text{and}$$

$$qV = \frac{2 \text{ GH VV} + \text{GH LV}}{2N}$$

where pL and qV are the expected frequencies of L and V alleles; GH LL, GH LV and GH VV are the number of animals with different genotypes. N is the number of genotyped animals.

The expected frequencies of the three genotypes were calculated as follows: GH LL = $n \times p^2$; GH VV = $n \times q^2$; GH LV = $2n \times p \times q$.

On the basis of the Hardy-Weinberg formulas, the expected frequencies of L and V alleles were 0.52 and 0.48 respectively.

The expected frequencies of the three genotypes were 27.50% (LL), 49.88% (LV) and 22.62% (VV). The

observed number of genotypes were 21.95% (LL), 60.98% (LV) and 17.07% (VV). The calculated χ^2 value was 8.11 (d.f. = 1), indicating Hardy-Weinberg disequilibrium in the population (Table 2). Comparison of the observed with expected numbers of GH genotypes showed a deficit of both Leu/Leu and Val/Val homozygotes and consequently an excess of Leu/Val heterozygotes ($p \leq 0.005$).

These animals are not relatives, not herdmates, they have different fathers and mothers as well, and they are even kept on different farms.

Sørensen et al. (2002) reported an almost equal frequency for the Leu and Val alleles in the same breed (0.51 and 0.49), indeed Lucy et al. (1993) reported a value of 0.56 and 0.44 for alleles Leu and Val respectively. To our knowledge there has been breed variation in the allele frequency for this locus and, in particular, a low Val frequency was reported in Holsteins (0.07 Lucy et al., 1993; 0.08 Vukasinovic et al., 1999; 0.09 Sabour et al., 1997), Guernsey (0.08 Lucy et al., 1993) and Bavarian and Tyrolean Brown (0.10 Schlee et al., 1994), while the Brown Swiss was monomorphic for the Leu allele (Lucy et al., 1993).

Effects on milk production traits

Table 3 shows least square means and their standard error for daily milk yield (kg/d), fat and protein yield (kg/d and percentage) and lactation length (d) for each of the GH genotypes. In particular, cows with the Leu/Leu genotype had a higher ($p < 0.05$) daily milk yield than those with the Val/Val genotype (+4.29 kg/d); this difference ($p < 0.01$) was even greater (+5.80 kg/d) when it was compared to Leu/Val heterozygotes. At the same time the daily yield of milk constituents was higher in Leu/Leu cows than in the other

genotypes. In particular, those with the Leu/Leu genotype showed a slightly significant advantage ($p < 0.05$) compared to cows with the Leu/Val genotype which had a higher fat yield (0.94 kg/d vs. 0.84 kg/d). A significant effect was found on the daily protein yield for the Leu/Leu genotype, this being significantly associated ($p < 0.01$) with a higher content than in the Leu/Val (+0.11 kg/d) and Val/Val (+0.09 kg/d) cows. When this difference was referred to fat and protein content, it was inverted since the cows with the Leu/Val genotype were characterized with a significantly higher percentage ($p < 0.05$). Finally, the lactation length of the Jersey cows was not affected by the GH polymorphism since all the cows were dried off when their lactation length ranged from 10-11 months.

Other authors had previously observed a significant dominance effect of GH genotypes on milk, fat and protein yields proving that the presence of the *Leu* allele affects milk quality traits, even if the results of these studies are not always in agreement. In particular, Lucy et al. (1993) showed that Holstein-Friesian cows homozygous for Leu-127 of bGH produced more milk than LV animals. Lee et al. (1996) determined that genetic merit (for EBV-milk and AYD-milk) was decreased in the presence of the Val-127 allele of the *GH* gene. Dybus (2002) found statistical differences between individuals of different genotypes in milk, fat and protein yield only in the first lactation: the cows of the *LL* genotype produced more milk (+225 kg), fat (+7 kg) and protein (+7 kg) than the *LV* individuals ($p \leq 0.01$).

Vukasinovic et al. (1999) found a significant allele substitution effect of the polymorphism on milk protein percentage in Holstein cows. Yao et al. (1996) did not find any significant effects associated with the polymorphism at codon 127 in the same breed.

These results indicate that the GH-AluI polymorphism in Italian Jersey cows may be related to a higher milk, fat, and protein yield. Nevertheless, the influence of the Val127 allele of the GH gene on the fat and protein content of milk should be verified by further studies.

CONCLUSIONS

The molecular techniques applied to genetics in conjunction with animal breeding techniques could give greater genetic gain by determining the potential of an animal, even before it is expressed phenotypically. A genetic marker serves the purpose of favourably relating alleles for quantitative characteristics with information about the individual mode of action and the interaction of genes, helping to understand the quantitative variations and their practical use in animal husbandry. According to Haley (1995), DNA markers present two possible future applications in animal selection, the combination of the best

alleles of two or more breeds and the selection of the best alleles within a breed or lineage.

Two alleles that generated three patterns were distinguished for the bGH gene in Italian Jersey cows. The Val/Val pattern was less frequent than the Leu/Leu or Leu/Val patterns. The significant superiority of the Leu/Leu genotype over the Val/Val and Leu/Val genotypes was observed for milk, fat and protein yield while the fat percentage was lower because of the higher milk yield.

Probably the observed differences could have resulted from another source of variation as reported by Aston et al. (1991); they determined that the fragment of somatotropin between amino acid 120 and 140 had lactogenic and somatogenic action, and, although that region did not take part in binding of growth hormone with its receptors, the interaction between the four α -helices could influence the structure of somatotropin (Chou and Zheng, 1992). It is supposed that bovine GH with Ala at the N terminus (191 aa) and Leu127 stimulate the release of IGF-I more than other variants of bGH (Vanderkooi et al., 1995). Schams et al. (1991) determined that a higher concentration of GH in blood was linked to a smaller concentration of IGF-I and higher milk yield. These observations are of economic interest, even though it is our assumption that other mutations within the GH gene, or genes closely linked to the polymorphic GH, could be responsible for this effect. Summarising, it appears that the results obtained in this study have not unequivocally indicated which allele of *GH* gene should be promoted in improvement of milk production traits.

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