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Effect of *hairless* gene polymorphism on the breeding values of milk production traits in Valle del Belice dairy sheep

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ABSTRACT

The aim of this work was to assess the association between the hairless genotypes and estimated breeding values (EBVs) for milk yield (MY), fat (FAT) and protein (PRT) content in Valle del Belice dairy sheep breed. A data set from 465 randomly chosen unrelated individuals was analyzed. EBV for MY, FAT and PRT contents were estimated by REML analysis of a single trait repeatability animal model. The genotype effect on EBV was assessed by ANOVA and by the Tukey–Kramer multiple comparison test. The PCR-SSCP test showed the presence of CC and CT genotypes in Valle del Belice individuals. Some differences in milk production traits between the genotypes were found. For MY, individuals with CT genotype produced 1.5 times more daily milk than CC homozygotes. Individuals with CC genotype showed eight times less FAT content and 1.7 times less PRT content than the heterozygous. However, these differences were not statistically different, probably due to the low frequency of the CT genotype. Considering our results, polymorphisms of the *hr* gene do not directly influence production traits, but if further studies confirm our hypothesis, the *hr* gene could be used in a marker assisted selection program.

1. Introduction

Advancements in the identification of loci and chromosomal regions affecting traits of economic interest open opportunities for the improvement of milk production traits in dairy breeds. Gene-assisted selection, that is, the use of functional mutations responsible for differences in phenotypes, is one of the options for marker-assisted selection (Dekkers, 2004). Loci with known effects on physiology of milk production have been proposed as candidate genes and relationships between their polymorphisms and several dairy traits have been studied (Grisart et al., 2002; Hayes and Goddard, 2001; Khatib et al., 2007, 2008; Macciotta et al., 2008).

The *hairless* (*hr*) gene is responsible of congenital hypotrichosis. This disorder in mammalian species results in partial or complete absence of the hair coat at birth. Concerning livestock species, cases of hypotrichosis have been found in cattle (Barlund et al., 2007; Bracho et al., 1984; Drögemüller et al., 2002; Marron, 2012; Mohr and Wriedt, 1928), pigs (Fernández et al., 2003; Lemus-Flores et al.,. 2001) and sheep, particularly in Australian Poll Dorset (Dolling and Brooker, 1966; Mackie and McIntyre, 1992), Karakul (Nel, 1964), Russian (Lazovskii, 1983), and Valle del Belice (Finocchiaro et al., 2000) sheep breeds. In humans, mice and rats, the *hr* genes are highly homologous, suggesting high conservation among mammals (Ahmad et al., 1998; Panteleyev et al., 1999). The protein encoded by this gene is a transcriptional co-repressor for thyroid hormone receptors (Potter et al., 2001) and, presenting a single zinc finger domain, it could probably belong to the family of transcription factors that bind to a core GATA motif.

Considering that the *hr* gene could be chosen as a candidate gene for the congenital hypotrichosis, several studies have been carried out for the Valle del Belice dairy

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sheep breed. In a preliminary study, Finocchiaro et al. (2000) reported the genetic control of the disorder as a Mendelian recessive trait and without concomitant chromosomal rearrangements. Subsequently, Finocchiaro et al. (2003a) sequenced the *hr* gene (GenBank Acc. No. AY130969) in the Valle del Belice sheep breed and have reported that a single nucleotide polymorphism (SNP), in particular a C→T transition at position 1312 bp in exon 3, caused an amino acid change at position 438 (Gln/Stop) of the mature protein. The T nucleotide, resulting in the TAG stop codon and in homozygote condition, has been associated with hypotrichotic phenotype in Valle del Belice sheep breed. Recently, the ovine *hr* gene has been physically mapped on chromosome 2 using fluorescence in situ hybridization (FISH) by Finocchiaro et al. (2008).

For Valle del Belice sheep farmers, hypotrichotic phenotype is considered as an undesirable defect; therefore, affected lambs are slaughtered soon after birth. The appearance of this disorder is probably due to the local farmers' tendency towards mating related animals.

Although the farmers know which animals are responsible for the appearances of the hypotrichotic phenotype, they prefer to maintain these ewes and rams within the farm and use them as sires and dams. Indeed, the farmers believe that rams, assumed to be carriers of the recessive allele after considering the pedigree, have female offspring with a higher milk production (Finocchiaro et al., 2003b).

Many economically important traits, including the dairy performance of sheep and goats, are polygenic in nature, which means that a great number of loci are responsible for the expression of the phenotype (Moioli et al., 1998). Genes influencing polygenic traits are difficult to identify and mutations in their sequences may alter animal performances as well as their breeding values (Madeja et al., 2004).

In Sicily, dairy sheep production represents an important resource for the development of the economies of hill and mountain areas, in which other economic activities are difficult to develop (Scintu and Piredda, 2007), and increased production of milk provides more revenue.

The aim of this work was to assess whether there is an association between the *hr* genotype and estimated breeding values (EBVs) for milk yield (MY), fat (FAT) and protein (PRT) contents in Valle del Belice dairy sheep breed.

2. Materials and methods

2.1. DNA samples and genotyping

A total of 465 randomly chosen unrelated individuals of Valle del Belice sheep breed were studied. All the individuals had a normal phenotype. Blood samples were collected using Vacutainer tubes containing EDTA as anticoagulant. Genomic DNA was extracted from buffy coats of nucleated cells using a salting out method (Miller et al., 1988). The PCR-SSCP (Polymerase Chain Reaction-Single Strand Conformation Polymorphism) protocol developed by Finocchiaro et al. (2003a) was used to detect the genotype (CC or CT) at position 1312 bp in exon 3 of the *hr* gene.

2.2. Milk samples and analysis

Phenotypic data were collected from 2000 to 2010 in three flocks of Valle del Belice dairy sheep located in the Agrigento province. Milk samples were collected at approximately monthly intervals, following an A4 recording scheme (ICAR, 2003). The first test day (TD) record was performed 31 days post-lambing. All ewes were milked manually twice a day and milk from morning and evening milking was collected to determine daily MY, PRT and FAT contents. The original dataset used for this study included 8732 TD records of 1644 lactations belonging to 465 ewes. Records were removed from the dataset when information of lambing day, litter size, number of lactation and TD for MY, FAT and PRT contents were missed. Moreover, records were removed when the genealogical information of the animal was not available. TDs were grouped into monthly classes of days in milk, ranging from 1 to 10 classes. Only records of animals with at least three TD records within a lactation were kept. After editing, the dataset consisted of 8156 TD records of 1564 lactations belonging to 436 ewes, daughters of 55 sires and 426 dams. Pedigree information for animals with records was traced back two generations. Maximum sizes of paternal and maternal families were 100 and 8, respectively.

2.3. Statistical analysis

Allelic and genotypic frequencies of the *hr* gene were calculated using the POPGENE software v1.32 (Yeh and Yang, 1999). A χ^2 test was performed to determine the Hardy–Weinberg equilibrium.

Preliminary analysis using the general linear model (GLM) procedure of SAS statistical package, were performed to determine the significance of the fixed effects where the conditional F-tests are implemented in the form of the ANOVA method (SAS Institute Inc., 2010). A single trait repeatability TD animal model was performed to estimate the EBV as follows:

$$y = Xb = Za + W_1 pe_1 + W_2 pe_2 + e$$

where y is the observation vector for TD of MY, FAT or PRT content as weighted mean of morning and evening milking, where weighting is according to the corresponding milk yield; b is the vector of fixed effects that includes herd year season (*HYS* 60 classes: three herds, 10 years and two seasons of birth from January to June and from July to December); herd test day (*HTD* 273 classes of test day within herd); *age* is the time-independent fixed effect of the age at first lambing class (four classes: 1 when first lambing occurred at 10–14 mo of age, 2 at 15–19 mo of age, 3 at 20 to 24 mo of age and 4 at 25–29 mo of age); when age at first lambing was missed, such as when ewes were recorded from a second or later lactation, the age at first lambing was approximated by considering a fixed interval between parities equal to 365 d; Legendre polynomial of order five (*op*: from 1 to 5), as used by Tolone et al. (2007) in the same dairy sheep breed to fit the lactation curve within parity class; a is the vector of direct additive genetic effects; pe_1 and pe_2 are the vectors of permanent environmental effects within and between

lactations, respectively. X, Z, W_1 and W_2 are the corresponding incidence matrices relating records to fixed, animal, permanent environmental within and between lactations effects, respectively.

The assumptions regarding these components were:

$$E\begin{bmatrix} y \\ a \\ pe_1 \\ pe_2 \\ e \end{bmatrix} = \begin{bmatrix} Xb \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}$$

and

$V(a) = A\sigma_a^2$
$V(pe_1) = A\sigma_{pe_1}^2$
$V(pe_2) = A\sigma_{pe_2}^2$
$V(e) = 1\sigma_e^2 = R$

where A is the numerator relationship matrix based on pedigree, and I and R are the identity and a residual matrices, respectively.

Breeding values for MY, FAT and PRT contents of the animals were estimated by the single repeatability animal model through REML method using ASReml 3 (Gilmour et al., 2009). The EBVs of the whole dataset were used to test association between milk production traits and the *hr* genotype. The effect of genotype on ewes EBV was tested for the three traits by analyzing least square means within the one-way ANOVA using R software (R Development Core Team, 2011). Moreover, the Tukey–Kramer multiple comparison test was used to analyze significance of differences between groups.

3. Results and discussion

The PCR-SSCP analysis showed the presence of the genotypes CC and CT. The TT genotype was absent having no sampled individuals with hypotrichotic phenotype. The frequencies of genotypes CC and CT were 0.929 and 0.071, respectively, while the frequencies of alleles C and T were 0.964 and 0.036, respectively. The population was in Hardy–Weinberg equilibrium ($\chi^2 = 0.610$; df = 1). The obtained frequencies were similar to the ones reported by Finocchiaro et al. (2003b). It was not possible to compare our results with other sheep breeds given the absence of similar studies in literature.

The descriptive statistics for milk production traits are reported in Table 1. Milk production was higher than that reported by Riggio et al. (2007) (1167 g), although they studied milk production in primiparous ewes, but lower than the observed by Cappio-Borlino et al. (1997) for the same sheep breed (1660 g). FAT and PRT contents were higher than those reported by the same authors, which found values of 68 g L^{-1} and 69 g L^{-1} for FAT, and values of 54.8 g L^{-1} and 51.8 g L^{-1} for PRT, respectively.

In Table 2 are reported the descriptive statistics for the EBVs of MY, FAT and PRT. These results showed an indirectly selection, within the farms, of Valle del Belice individuals that have higher MY than FAT and PRT content, because of the negative genetic correlation between these traits. It was difficult to compare our results on EBVs and accuracies with previous studies because different dataset and models were used to estimate the genetic parameters and no previous estimates for fat or protein EBVs were calculated in Valle del Belice breed. Moreover, the accuracy of EBVs for milk yield was lower than that reported by Portolano et al. (2009) who found an accuracy of 0.73. This was probably due to the larger dataset used by these authors, about twenty eight thousands observations. Moreover, they used a sire model instead of animal model making comparison of results more difficult. Moreover, the low accuracy values reported in the present study could be due to the poor structure of the pedigree of Valle del Belice sheep breed. This breed is, indeed, characterized by natural mating and relationship among rams and ewes are often uncertain.

The effect of the *hr* genotypes on milk traits EBV are reported in Table 3. Some differences were observed between the two genotypes, although these differences were not significantly different from 0. This result is probably due to the small number of animals carrying the CT genotype. The estimated genotype effect was positive for MY, whereas it was negative for FAT and PRT content. For MY, individuals with CT genotype produced 1.5 times more daily milk than CC homozygotes. Individuals with CC genotype showed eight times less FAT content, and almost 1.7 times less PRT content than the heterozygote genotype.

Table 1
Descriptive statistics for milk production traits.

Trait	Mean ± SD	Min.	Max.
MY (g)	1319 ± 557	117	4140
FAT (g L^{-1})	72.27 ± 11.29	30.00	133.90
PRT (g L^{-1})	58.83 ± 6.87	21.70	93.30

SD: standard deviation.

Table 2
Descriptive statistics for estimated breeding values (EBV) of milk production traits.

Trait	EBV ± SD	1st Qu.	3rd Qu.	*r*	1st Qu.	3rd Qu.
MY (g d^{-1})	29.18 ± 65.45	−17.34	73.24	0.49	0.46	0.53
FAT (g L^{-1})	−0.15 ± 0.53	−0.45	0.18	0.35	0.31	0.40
PRT (g L^{-1})	−0.15 ± 0.75	−0.60	0.38	0.47	0.44	0.51

SD: standard deviation, 1st and 3rd Qu.: first and third quantiles, *r*: accuracy.

Table 3
Least squares means depicting the effect of hr genotype on milk production traits.

Trait	Genotype	Estimated genotype effect	SE
MY (g d^{-1})	CC	28.075[a]	± 3.241
	CT	43.565[a]	± 12.160
FAT (g L^{-1})	CC	−0.162[a]	± 0.027
	CT	−0.020[a]	± 0.086
PRT (g L^{-1})	CC	−0.154[a]	± 0.038
	CT	−0.089[a]	± 0.124

[a] $p > 0.05$

Considering our results, there is no evidence of a direct influence of the *hr* gene polymorphisms on production traits. However, it can be hypothesized that this gene, mapped on chromosome 2, is close to an unknown milk production QTL in sheep. This hypothesis is supported by the identification of a suggestively significant QTL on chromosome 2 for protein and fat percentage in Churra sheep (Gutierrez-Gil et al., 2009).

In summary, no association was found between the *hr* gene polymorphisms and milk yield, fat and protein content, probably due to the low frequency of CT genotype. Further studies will be conducted in order to collect more heterozygote CT individuals of Valle del Belice sheep breed and to set up an experimental design focused on identification of QTL. If further studies confirm our hypothesis, the *hr* gene could be used in programs based on marker assisted selection.

Conflict of interest

The authors declare no conflict of interest.

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