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SHORT COMMUNICATION

Investigation of allele frequencies of the growth hormone receptor (GHR) F279Y mutation in dairy and dual purpose cattle breeds

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ABSTRACT

A major QTL for milk production traits was reported in the middle of bovine chromosome 20 and, for its map position, the growth hormone receptor (GHR) gene was considered a strong positional and functional candidate gene. A missense mutation in exon VIII (F279Y amino acid substitution) showed highly significant effects mainly on milk composition (protein percentage and fat percentage) as well as on milk yield in several dairy cattle populations. As no information about the frequency of these two GHR alleles is available in any population, we studied their distribution in dairy and dual purpose cattle breeds reared in Italy. A total of 679 animals belonging to seven cattle breeds (Italian Holstein-Friesian, n=108; Italian Brown, n=104; Italian Simmental, n=104; Jersey, n=104; Reggiana, n=108; Modenese, n=66; Rendena, n=85) were sampled. A new PCR-RFLP protocol was designed to analyse this mutation inserting an artificial restriction site for enzyme SspI. In all investigated breeds, allele F was the most frequent and ranged from 0.947 (Italian Brown and Jersey) to 0.727 (Italian Holstein-Friesian). In Rendena, Italian Simmental, Reggiana and Modenese it was 0.824, 0.909, 0.921 and 0.924, respectively. For all breeds no significant deviation from the Hardy-Weinberg equilibrium was observed. Differences in allele frequencies were statistically significant between the Italian Holstein Friesian and Rendena against all other breeds. Due to the high frequency of the putative positive allele for milk protein percentage the use of the GHR F279Y marker in marker assisted selection plans should not have a great impact on this trait in the studied breeds.

Key words: Cattle breeds, Growth hormone receptor, Mutation, Allele frequency, Milk production.

RIASSUNTO

STUDIO DELLE FREQUENZE ALLELICHE DELLA MUTAZIONE F279Y NEL GENE GHR IN ALCUNE RAZZE BOVINE DA LATTE E A DUPLICE ATTITUDINE

Una mutazione puntiforme nell’esone 8 del gene del recettore dell’ormone della crescita (GHR), che cambia l’amminoacido fenilalanina in tiro sina in posizione 279 (F279Y), è stata indicata avere un effetto positivo sulla percentuale di proteina del latte (allele F) o sulla quantità di latte (allele Y). Fino ad ora nessun dato relativo alla distribuzione di questi due alleli era disponibile in alcuna razza bovina e per questo nel presente studio abbiamo analizzato le frequenze alleliche di questo polimorfismo in alcune razze bovine da latte e a duplice attitudine.
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latte e a duplice attitudine allevate in Italia. Campioni di latte, peli e seme sono stati colletti da 679 animali appartenenti a sette razze diverse (Frisona Italiana, n=108; Bruna Italiana, n=104; Pezzata Rossa Italiana, n=104; Jersey, n=104; Reggiana, n=108; Modenese, n=66; Rendena, n=85). Per l’analisi della mutazione F279Y del gene GHR è stato messo a punto un protocollo basato sulla tecnica di PCR-RFLP con un primer forward modificato, che inserisce nel frammento di DNA amplificato un sito di taglio artificiale per l’enzima di restrizione SspI. Per confermare i risultati dell’analisi PCR-RFLP, è stato sequenziato un frammento del gene di 381 pb ottenuto da bovini con diverso genotipo. In tutte le razze analizzate l’allele F è risultato il più frequente con un valore massimo di circa il 95% nella Bruna Italiana e nella Jersey ed un valore minimo di circa il 73% nella Frisona Italiana. Nelle razze Rendena, Pezzata Rossa Italiana, Reggiana e Modenese la frequenza di questo allele è risultato, rispettivamente dell’82%, 91%, 92% e 92%. I dati ottenuti non hanno rilevato alcun scostamento statisticamente significativo dall’equilibrio di Hardy-Weinberg. Le frequenze alleliche della Frisona Italiana e della Rendena sono risultate essere significativamente (rispettivamente P<0,01 e P<0,05) diverse da quelle delle altre razze. Data l’elevata frequenza dell’allele F l’utilizzo del polimorfismo al locus GHR in piani di selezione assistita da marcatori non dovrebbe avere un notevole impatto per il miglioramento della percentuale di proteina nelle razze studiate.

Parole chiave: Razze bovine, GHR, Mutazione del DNA, Frequenze alleliche, Produzione latte.

Introduction

Several studies have indicated that bovine chromosome 20 (BTA20) harbours quantitative trait loci (QTL) for milk yield, milk composition, functional and reproductive traits (i.e.: Arranz et al., 1998; Viitala et al., 2003; Ashwell et al., 2004; Fontanesi et al., 2006).

A combined linkage and linkage disequilibrium analysis positioned a major QTL for milk production traits in the middle of BTA20 and, for its map position, the growth hormone receptor (GHR) gene was considered a strong positional and functional candidate gene (Blott et al., 2003), knowing the major role played by the growth hormone axis in the initiation and maintenance of lactation (Parmentier et al., 1999). In this gene several mutations have been located in the regulatory region, 5'-untranslated region (UTR), 3'-UTR and in the coding sequence (i.e.: Moisio et al., 1998; Aggrey et al., 1999; Blott et al., 2003; Viitala et al., 2006). A single nucleotide polymorphism in exon VIII, a T>A transversion, that causes the replacement of an amino acid, a phenylalanine (F) to tyrosine (Y) substitution, in a highly conserved transmembrane domain of the GHR protein at position 279 (F279Y mutation), showed highly significant effects mainly on milk composition (protein percentage and fat percentage) as well as on milk yield in Dutch and in New Zealand Holstein as well as in New Zealand Jersey populations (Blott et al., 2003). Allele F was indicated to increase the percentage of protein and fat in the milk but with a negative effect on milk yield. On the other hand, allele Y was suggested to have a positive effect on milk yield and a negative effect on protein and fat percentage. At least in part, these effects have been indirectly confirmed in the Italian Holstein population (Fontanesi et al., 2006) and observed in Finnish Ayrshire (Viitala et al., 2006). Thus, it seems that the effects of this mutation are consistent across breeds and populations, even if the exact mechanism by which the F279Y amino acid change exerts influence on milk production traits is still unclear (Zhou and Jiang, 2006). In any case, it should be pointed out that other putative QTL affecting milk production traits on BTA20 may be close to the GHR gene (Fontanesi et al., 2006; Viitala et al., 2006).

However, the GHR F279Y mutation has
been included or is currently evaluated for its inclusion in ongoing marker assisted selection (MAS) programs started for commercial purposes in a few countries (Dekkers, 2004; Druet et al., 2006).

So far this polymorphic site has been analysed only in few populations that were used for QTL analysis (Blott et al., 2003; Viitala et al., 2006) but no information is available about the frequency of the two alleles in any Italian cattle population.

Here we studied the distribution of these two GHR alleles in several cattle breeds reared in Italy, as a first step to plan studies to evaluate their effects and to investigate their possible use in MAS.

**Material and methods**

Milk, hair or semen were sampled from a total of 679 animals belonging to seven cattle breeds: Italian Holstein-Friesian, n=108; Italian Brown, n=104; Italian Simmental, n=104; Jersey, n=104; Reggiana, n=108; Modenese, n=66; Rendena, n=85 (Table 1).

A PCR-RFLP method was set up to analyse the T>A point mutation of exon VIII of the GHR gene. Primers (forward: AAT-ACTTGGGCTAGCAGTGA; reverse: ACGTTTCAGCGGTTGAGTA) were designed on sequences AAFC01123550 and CQ817600.

PCR was performed using a PT-100 (MJ Research, Watertown, MA, USA) thermal cycler in a final volume of 20 µl containing the DNA template (about 10-100 ng), 1 U DNA EuroTaq DNA polymerase (EuroClone Ltd., Paington, Devon, UK), 1X PCR Buffer, 2.5 mM dNTPs, 10 pmol of each primer and 2.0 mM of MgCl₂. PCR was carried out using

**Table 1. Genotype and allele frequencies of the GHR F279Y mutation in the investigated cattle breeds.**

| Breeds                | N. of animals* | Genotype frequency (n. of animals) | Allele frequency |
|-----------------------|---------------|-----------------------------------|------------------|
|                       |               | FF (n) | FY (n) | YY (n) | F (n) | Y (n) | Diff.# |
| Italian Holstein Friesian | 108          | 0.509 (55) | 0.435 (47) | 0.056 (6) | 0.727 | 0.273 | A/d |
| Italian Brown         | 104          | 0.894 (93) | 0.106 (11) | 0.000 (0) | 0.947 | 0.053 | B/D |
| Italian Simmental     | 104          | 0.827 (86) | 0.163 (17) | 0.010 (1) | 0.909 | 0.091 | B/d |
| Jersey                | 104          | 0.894 (93) | 0.106 (11) | 0.000 (0) | 0.947 | 0.053 | B/D |
| Rendena               | 85           | 0.682 (58) | 0.282 (24) | 0.036 (3) | 0.824 | 0.176 | b/C |
| Reggiana              | 108          | 0.880 (95) | 0.083 (9)  | 0.037 (4) | 0.921 | 0.079 | B/d |
| Modenese              | 66           | 0.848 (56) | 0.152 (10) | 0.000 (0) | 0.924 | 0.076 | B/d |

* Italian Holstein Friesian, 17 sires and 91 cows; Italian Brown, 104 cows; Italian Simmental, 104 cows; Jersey, 2 sires and 102 cows; Rendena, 85 cows; Reggiana, 108 sires; Modenese, 21 sires and 45 cows.

# Statistical levels of differences in allele frequencies between breeds: comparisons between the Italian Holstein Friesian and the other breeds, A,B P<0.001, A,b P<0.05; comparisons between the Rendena and the other breeds, C,D P<0.001, C,d P<0.01, C,d P<0.05.
the following profile: 5 min at 95°C; 35 amplification cycles of 30 s at 95°C, 30 s at 60°C, 30 s at 72°C; 10 min at 72°C.

Primer forward inserts an artificial restriction site for SspI by means of a mismatched base (underlined base in the primer sequence). Restriction analysis was carried out overnight at 37°C in a total volume of 25 µl containing 5 µl of the PCR product, 5 U of SspI, 1X reaction buffer. All digested product was electrophoresed in 10% polyacryamide:bisacrylamide 29:1 TBE 1X gels. DNA fragments were visualized with ethidium bromide.

The genotypes obtained from a few animals were confirmed by sequencing of the region containing the polymorphism. PCR primers (forward: 5’-CAGATTCCAGTTTCCATGGTT-3’; reverse: 5’-TGCAGACTTTTAAAGGTCAT-3’) designed on the reported sequence entries, were used to amplify genomic DNA of sires showing the 3 different genotypes using the PCR-RFLP protocol. The PCR conditions were as reported above. Only the annealing temperature changed and was set up at 58°C. The obtained fragments of 361 bp were sequenced, as described in Russo et al. (2007), on both strands using the same PCR primers.

A χ² contingency test was used to evaluate differences in allele frequencies between the breeds.

Results and discussion

To genotype the T>A missense mutation within exon VIII of the GHR gene, a new genotyping protocol, a simple PCR-RFLP test, was developed in this study to substitute the oligonucleotide ligation assay designed by Blott et al. (2003). As this mutation does not create/disrupt any recognised site for any restriction enzyme an artificial restriction site for enzyme SspI was inserted in the amplified DNA fragment by means of a mismatched base in the forward primer. Then, after digestion of the 182 bp amplicon, two DNA fragments (158 and 24 bp) are produced when base T is present (allele F), while when the alternative base (A) is present (allele Y), the amplified DNA fragment does not contain any recognition site for SspI, thus no restriction is produced. Sequencing of the 361 bp amplicon of the GHR gene containing the polymorphic site obtained from DNA samples that have been genotyped using the described PCR-RFLP protocol confirmed the obtained results.

Allele and genotype frequencies observed in the analysed breeds are reported in Table 1. In all investigated breeds, allele F was the most frequent and ranged from 0.947 (Italian Brown and Jersey) to 0.727 (Italian Holstein-Friesian). In Rendena, Italian Simmental, Reggiana and Modenese it was 0.824, 0.909, 0.921 and 0.924, respectively. Frequency of the FF genotype varied between breeds ranging from 0.894 (Italian Brown and Jersey) to 0.509 (Italian Holstein-Friesian). Genotype FY was observed with the highest frequency in Italian Holstein-Friesian (0.435) and the lowest in Reggiana (0.083). A few animals with genotype YY were identified in Italian Holstein (0.056), Reggiana (0.037), Rendena (0.036) and Italian Simmental (0.010). For all breeds no significant deviation from the Hardy-Weinberg equilibrium was observed for this polymorphism.

Differences in allele frequencies were statistically significant between the Italian Holstein Friesian and all other breeds (Italian Brown, Italian Simmental, Jersey, Reggiana and Modenese: P<0.001; Rendena: P<0.05). Rendena allele frequencies differed statistically from the frequencies observed in the other breeds (Italian Brown, Jersey: P<0.001; Reggiana: P<0.01; Italian Simmental, Modenese: P<0.05). No
difference was statistically significant between Italian Brown, Italian Simmental, Jersey, Reggiana and Modenese.

In a MAS program the relative frequency of the QTL alleles is of paramount importance. If the favourable allele(s) is (are) already at high frequency in the population under selection, then little can be gained from MAS while if the favourable allele(s) is (are) rare a larger impact can be obtained. This should be also true when a QTL is transformed in a quantitative trait gene (QTG), as may be considered the case of the GHR gene, and a direct marker is available for this purpose (Dekker, 2005). Considering F the favourable allele, due to the suggested positive effect on milk protein percentage, a MAS program aimed to increase protein percentage based on the GHR F279Y polymorphic site could be more effective in the Italian Holstein Friesian than in the other analysed breeds. However, the ~73% of allele F observed in the Italian Holstein Friesian is still a high frequency that is not very favourable for MAS as discussed in several simulation studies (i.e.: Kashi et al., 1990; Schulman et al., 1999).

Thus, the use of this marker in MAS should be evaluated with caution considering also the complex breeding goals of the analysed breeds, their population structure as well as the presence of other close QTL or other mutations in this gene affecting production traits. Actually, other investigations have indicated that the prolactin receptor gene, positioned a few cM from the GHR gene on BTA20 (Viitala et al., 2006) may affect milk production traits in dairy cattle. Moreover, in beef cattle other mutations in the GHR gene have been indicated to be associated with several meat production traits (Ge et al., 2003; Curi et al., 2005; Di Stasio et al., 2005). It could be interesting to study the frequencies of these additional polymorphisms and reconstruct haplotypes for this gene as well as to evaluate their effects also in dairy cattle breeds.

Conclusions

The results showed that in all investigated breeds both F and Y alleles are present at the GHR locus even if the former is always the most frequent. Due to the high frequency of the F allele the use of the GHR F279Y marker in MAS plans should not have a great impact for the improvement of milk protein percentage in the studied breeds.

REFERENCES

Aggrey, S.E., Yao, J., Sabour, M.P., Lin, C.Y., Zadworny, D., Hayes, J.F., Kuhnlein, U., 1999. Markers within the regulatory region of the growth hormone receptor gene and their association with milk-related traits in Holstein. J. Hered. 90:148-151.

Arranz, J.J., Coppieters, W., Berzi, P., Cambisano, N., Grisart, B., Karim, L., Marcq, L., Merez, C., Riquet, J., Simon, P., Vanmanshoven, P., Wagenaar, D., Georges, M., 1998. A QTL affecting milk yield and composition maps to bovine chromosome 20: a confirmation. Anim. Genet. 29:107-115.

Ashwell, M.S., Heyen, D.W., Sonstegard, T.S., Van Tassell, C.P., Da, Y., VanRaden, P.M., Ron, M., Weller, J.I., Lewin, H.A., 2004. Detection of quantitative trait loci affecting milk production, health, and reproductive traits in Holstein cattle. J. Dairy Sci. 87:468-475.

Blott, S., Kim, J.J., Moisio, S., Schmidt-Küntzel, A., Cornet, A., Berzi, P., Cambisano, N., Ford, C., Grisart, B., Johnson, D., Karim, L., Simon, P., Snell, R., Spelman, R., Wong, J., Vilikki, J., Georges, M., Farnir, F., Coppieters, W., 2003. Molecular dissection of a quantitative trait locus: a phenylalanine-to-tyrosine substitution
in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. Genetics 163:253-266.

Curi, R.A., Oliveira, H.N., Silveira, A.C., Lopes, C.R., 2005. Effects of polymorphic microsatellites in the regulatory region of IGF 1 and GHR on growth and carcass traits in beef cattle. Anim. Genet. 36:58-62.

Dekkers, J.C.M., 2004. Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. J. Anim. Sci. 82 (E. Suppl.):E313-E328.

Di Stasio, L., Destefanis, G., Brugiapaglia, A., Albera, A., Rolando, A., 2005. Polymorphism of the GHR gene in cattle and relationships with meat production and quality. Anim. Genet. 36:138-140.

Druet, T., Fritz, S., Boichard, D., Colleau, J.J., 2006. Estimation of genetic parameters for quantitative trait loci for dairy traits in the French Holstein population. J. Dairy Sci. 89:4070-4076.

Fontanesi, L., Russo, V., Scotti, E., Zambonelli, P., Dall’Olio, S., Bigi, D., Canavesi, F., Lipkin, E., Soller, M., Davoli, R., 2006. Analysis of bovine chromosome 20 for QTL affecting milk production and reproductive traits applying a milk selective DNA pooling strategy in the Italian Holstein population. Comm. 20-18 in CD-ROM Proc. 8th World Congr. Genet. Appl. Livest Prod., Belo Horizonte, Brazil.

Ge, W., Davis, M.E., Hines, H.C., Irvin, K.M., Simmen, R.C., 2003. Association of single nucleotide polymorphisms in the growth hormone receptor genes with blood serum insulin-like growth factor I concentration and growth traits in Angus cattle. J. Anim. Sci. 81:641-648.

Kashi, Y., Hallerman, E., Soller, M., 1990. Marker assisted selection of candidate bulls for progeny testing programs. Anim. Prod. 51:63-74.

Moisio, S., Elo, K., Kantanen, J., Vilkkii, J., 1998. Polymorphism within the 3’ flanking region of the bovine growth hormone receptor gene. Anim. Genet. 29:55-57.

Parmentier, I., Portetelle, D., Gengler, N., Prandi, A., Bertozzi, C., Vleurick, L., Gilson, R., Renaville, R., 1999. Candidate gene markers associated with somatotropic axis and milk selection. Domest. Anim. Endocrinol. 17:139-148.

Russo, V., Fontanesi, L., Scotti, E., Tazzoli, M., Dall’Olio, S., Davoli, R., 2007. Analysis of melanocortic 1 receptor (MC1R) gene polymorphisms in some cattle breeds: their usefulness and application for breed traceability and authentication of Parmigiano Reggiano cheese. Ital. J. Anim. Sci. 6:257-272.

Schulman, N.F., De Vries, M.J., Dentine, M.R., 1999. Linkage disequilibrium in two-stage marker-assisted selection. J. Anim. Breed. Genet. 116:99-110.

Viitala, S.M., Schulman, N.F., de Koning, D.J., Elo, K., Kinos, R., Virta, A., Virta, J., Mäki-Tanila, A., Vilkkii, J.H., 2003. Quantitative trait loci affecting milk production traits in Finnish Ayrshire dairy cattle. J. Dairy Sci. 86:1828-1836.

Viitala, S., Szyda, J., Bloot, S., Schulman, N., Lidauer, M., Mäki-Tanila, A., Georges, M., Vilkkii, J.H., 2006. The role of the bovine growth hormone receptor and prolactin receptor genes in milk, fat and protein production in Finnish Ayrshire cattle. Genetics 173:2151-2164.

Zho, Y., Jiang, H., 2006. Short Communication: A milk trait-associated polymorphism in the bovine growth hormone receptor gene does not affect receptor signaling. J. Dairy Sci. 89:1761-1764.