Variability in candidate genes revealed associations with meat traits in the Piemontese cattle breed

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Abstract

In the last years an increasing number of associations between single nucleotide polymorphisms (SNPs) in candidate genes and production traits have been reported in beef cattle, but very often the results were not validated and a few studies considered breeds homozygous for the allele responsible for the muscular hypertrophy. Therefore, we analysed the variability of 19 previously reported SNPs in 12 genes (GH, GHR, GDF8, GHRL, IGF2, LEP, LEPRT, MYF5, NPY, POMC, UCP2, UCP3) in the hypertrophic Piemontese breed and investigated the effects of the observed polymorphisms on growth and conformation. Fourteen SNPs were polymorphic and haplotype effects were estimated. Negligible effects on the investigated traits were observed for GHRL, MYF5, NPY, POMC, UCP2 and UCP3 genes. The GHR gene significantly affected daily gain and its effect was further increased when haplotypes were considered. The C allele at LEP-1 and LEP-2 had moderate negative effects on the considered traits, whereas the C allele at LEP-3 mostly had positive effects; haplotypes in the LEP gene showed weaker but favourable associations with all the traits. The C allele at IGF2 and LEPRT had favourable effects on daily gain and negative effects on meat conformation traits. The associations observed for GHR and LEP were consistent with those of previous studies, providing additional evidence of their usefulness as markers. Practical aspects of the applications to the breeding programme of the Piemontese breed need to be examined.

Introduction

To date a great number of candidate genes for production traits have been suggested in different livestock species, based on the knowledge of their position and/or function. For meat production, the interest has been mainly focused on genes involved in growth and meat quality, but only for a limited number of genes the effects of their polymorphisms have been investigated, often in a single breed. On the other hand, the recent development of high-density single nucleotide polymorphism (SNP) genotyping microarrays has opened new selection perspectives for the possibility of estimating the breeding value of animals with no phenotypic records, with the potential advantages of increased genetic gain and lower costs (Meuwissen et al., 2001). However, as many of the genotyped SNPs are located in anonymous regions, the detection of associations with traits of interest does not directly lead to the identification of the underlying genes (Magee et al., 2010). For these reasons the candidate gene approach, which aims at identifying specific polymorphisms responsible for the observed effects in genes biologically related to the traits of interest, is still a valuable strategy (Ron and Weller, 2007).

On the basis of these considerations we carried out the present study in order to give a contribution to the analysis of genes possibly related to meat production. We focused on 12 genes, which were selected on the basis of their biological functions and for which effects on the traits of interest had been reported: growth hormone (GH), growth hormone receptor (GHR), growth differentiation factor 8 (GDF8), ghrelin (GHRL), insulin-like growth factor 2 (IGF2), leptin (LEP), leptin receptor (LEPR), myogenic factor 5 (MYF5), neuropeptide Y (NPY), proopiomelanocortin (POMC), uncoupling protein 2 (UCP2), uncoupling protein 3 (UCP3). The products of most of these genes are involved in biologically related processes regulating feed intake and growth. Circulating leptin, after binding to specific receptors in the brain, exerts its effects on feed intake and energy homeostasis via neurotransmitters such as neuropeptide Y and proopiomelanocortin (Houseknecht and Portocarrero, 1998). Leptin also increases the expression of uncoupling protein 2 and 3, involved in energy expenditure (Scarpone et al., 1997), and modulates the secretion of growth hormone (Zieba et al., 2003), which binds to GH receptors on target tissues, activating the signal transduction culminating in GH biological effects (Kopchik and Andry, 2000). Polymorphisms in the considered genes have been shown to affect growth, feed efficiency and carcass quality in different cattle breeds and crossbreds (Kim et al., 2004; Li et al., 2004; Buchanan et al., 2005; Nkrumah et al., 2005; Di Stasio et al., 2007; Goodall and Schmutz, 2007; Devuyst et al., 2008; Sherman et al., 2008). It seems worth noting that very few studies considered breeds homozygous for the allele responsible for the muscular hypertrophy, which might interfere with genes affecting meat production as a consequence of its well known effects on growth and muscle development.

Therefore, the objective of this study was to estimate the variability of the above twelve genes and their associations with traits recorded during the performance testing of breeding candidates in the hypertrophic Piemontese breed.

Materials and methods

The study was carried out on 201 Piemontese male calves enrolled in the performance testing programme at the central Station of the Italian Association of Piemontese Cattle Breeders. The performance testing programme of the Piemontese breed is described in Albera et al. (2001). Eight traits recorded during the performance testing were...
considered: average daily gain (DG), withers width (WW), shoulder musculature (SM), loins width (LW), loins thickness (LT), thigh musculature (TM), thigh profile (TP) and bone thickness (BT). The conformation traits were graded through a linear scoring of live animals using a 9-point scale, as reported by Albera et al. (2001). Descriptive statistics for the investigated traits are presented in Table 1.

Blood samples were collected in tubes containing ethylenediaminetetraacetic acid as an anticoagulant and kept at 4°C until DNA isolation. Genomic DNA was extracted using the NucleoSpin® Blood kit (Macherey-Nagel, Düren, Germany). A total of 19 SNPs were investigated in 12 genes (Table 2). Genotyping was performed by a commercial company (LGC Genomics, Biotools, London, UK).

Allele frequencies were estimated by simple counting. Tests for Hardy-Weinberg equilibrium at each SNP and for linkage disequilibrium between the SNP pairs were performed using SPSS software (SPSS, 2002). For the linked SNPs, haplotypes were constructed using the PHASE v.2.1 program (Stephens et al., 2001), which implements a Bayesian method for reconstructing haplotypes from population genotype data.

The association of the observed polymorphisms with phenotypes for the recorded traits was investigated using a statistical model similar to that used for the prediction of breeding values of Piemontese bulls, but also including the effect of the single SNP or haplotype.

The general univariate linear model, in matrix notation, was:

\[ y = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Wc} + \mathbf{e} \]

where y is a vector of observations on the considered trait; b is a vector of systematic nongenetic effects; u is a vector of animal additive genetic effects; c is a vector of SNP genotype or haplotype effects; e is a vector of random residuals; X, Z and W are incidence matrices of proper order relating observations to b, u and c, respectively.

For all traits, nongenetic effects included in the linear model were the effect of the contemporary group of animals on test and of the parity of the dam. Additionally, the weight at the beginning of the test for growth and the weight at scoring for meat conformation traits were included as covariates. For the single SNPs analysis, the model included the effect of the SNP genotype, whereas for the haplotypes the regression on the number of haplotype copies was included, as the accuracy of the haplotype reconstruction was very high (P=0.938 to 0.998).

The effects of the observed polymorphisms were investigated using Bayesian procedures. The Bayesian analysis, performing numerical integration through Gibbs sampling, was used to estimate the marginal posterior distribution of parameters of concern (Legarra et al., 2008).

### Table 1. Descriptive statistics of the traits in the analysed sample.

| Trait                        | Mean  | sP    | Minimum | Maximum | sA |
|------------------------------|-------|-------|---------|---------|----|
| Average daily gain, g/d      | 1353.10 | 125.42 | 953.00  | 1705.00 | 76.1 |
| Withers width                | 7.04   | 1.01  | 4.67    | 9.00    | 0.56 |
| Shoulder musculature         | 7.00   | 0.99  | 4.00    | 9.00    | 0.52 |
| Loin width                   | 6.85   | 0.87  | 4.67    | 9.00    | 0.44 |
| Loin thickness               | 7.08   | 0.95  | 4.67    | 9.00    | 0.44 |
| Loin width                   | 7.43   | 1.04  | 4.67    | 9.00    | 0.75 |
| Thigh muscularity            | 7.20   | 1.05  | 4.33    | 9.00    | 0.76 |
| Bone thickness               | 6.05   | 0.94  | 5.03    | 8.00    | 0.51 |

sP, phenotypic standard deviation; sA, additive genetic standard deviation.

### Table 2. Single nucleotide polymorphism information.

| Gene   | Bovine chromosome | SNP name | SNP location | SNP description | Frequency of the first allele |
|--------|-------------------|----------|--------------|-----------------|-------------------------------|
| GDF8   | BTA2              | GDF8-1   | promoter     | AJ438578 g.843T>A | 1.00                          |
|        |                   | GDF8-2   | exon 1       | AF320998 g.430C>A | 1.00                          |
| GH     | BTA19             | GH       | promoter     | AJ458511 g.358C>T | 1.00                          |
| GHR    | BTA20             | GHR-1    | promoter     | U15731 g.907C>T   | 1.00                          |
|        |                   | GHR-2    | promoter     | AF126288 g.140A>G | 0.45                          |
|        |                   | GHR-3    | intron 4     | AV434977 g.302A>G | 0.65                          |
| GHR1   | BTA22             | GHR1     | intron 3     | AV559980 g.446A>G | 0.80                          |
| IGF2   | BTA29             | IGF2     | exon 2       | AV237543 g.150C>T | 0.75                          |
| LEP    | BTA4              | LEP-1    | promoter     | AB070368 g.528C>T | 0.58                          |
|        |                   | LEP-2    | promoter     | AB070368 g.1759G>C | 0.86                          |
|        |                   | LEP-3    | exon 2       | AV38588 g.305G>C  | 0.17                          |
| LEPR   | BTA3              | LEPR     | exon 20      | AV38588 g.115C>T | 0.92                          |
| MF5    | BTA5              | MF5      | intron 2     | M95064 g.1948A>G  | 0.42                          |
| NPY    | BTA4              | NPY-1    | intron 2     | AY910104 g.284A>G | 1.00                          |
|        |                   | NPY-2    | intron 2     | AY910104 g.666A>G | 0.23                          |
|        |                   | NPY-3    | intron 2     | AY910104 g.302C>T | 0.32                          |
| POMC   | BTA11             | POMC     | exon 3       | J00012 g.254G>C   | 0.83                          |
| UC2    | BTA15             | UC2      | intron 2     | AY147821 g.386G>C | 0.18                          |
| UCP3   | BTA15             | UCP3     | intron 3     | AF127004 g.1099G>A | 0.23                          |

SNP, single nucleotide polymorphism.
Animal and residual effects were assumed to be normally distributed a priori as

\[ u \sim N(0, \sigma^2_u) \] and \[ e \sim N(0, \sigma^2_e) \]

where \( A \) was the numerator relationship matrix, \( \sigma^2_u \) was the additive genetic variance; \( I \) was an identity matrix of proper order; \( \sigma^2_e \) was the residual variance.

Flat priors were assumed for systematic non-genetic and for SNP genotype or haplotype effects. As the number of animals included in the study was too limited to estimate variance components, estimates of additive genetic and residual variances obtained by Albera et al. (2001) were used. A single chain of 1,000,000 iterations with a burn-in of 200,000 was run for each trait/SNP analysis, saving samples every 400 iterations.

Inference on additive and dominance SNP effects, as defined by Falconer and Mackay (1996), was based on the estimated marginal posterior density of these effects. Haplotype effects were estimated as deviations from the effect of the reference haplotype which was arbitrarily set to zero. The mean of the marginal posterior distribution of a SNP/haplotype effect was used as a point estimate of the effect.

On the basis of the realised response to selection for meat traits in the Piemontese population in the last ten years (ANABORAPI, 2010) and also considering the effectiveness of exploiting variation due to candidate genes, a SNP/haplotype effect was considered to be relevant when its absolute value was greater than 10% of the additive genetic standard deviation of the trait. For a given effect, the probability of being relevant was calculated from the estimated marginal posterior distribution.

### Results

Genotyping revealed that GDF8-1, GDF8-2, GH, GHR-1 and NPY-1 were monomorphic in the examined sample (Table 2). The absence of variability for GDF8-2, in the exon 1 of GDF8 gene, seems noteworthy, because previous studies had reported the presence of the A allele in the Piemontese breed (McPherron and Lee, 1997), although at a very low frequency (0.02; Vankan et al., 2010). The polymorphic SNPs showed a different degree of variability, with the minor allele frequency ranging from 0.08 (LEPR) to 0.45 (GHR-2). For all the SNPs, the genotype frequencies were in agreement with Hardy-Weinberg equilibrium frequencies (P>0.05). A linkage disequilibrium significant at the 5% nominal level was observed for SNPs within a gene: GHR-2 – GHR-3, LEP-1 – LEP-2 – LEP-3, NPY-1 – NPY-2. For these SNPs, both single

### Table 3.

| Trait   | GHR-2 | GHR-3 | Haplotype effect |
|---------|-------|-------|------------------|
|         | a (A vs G) | d | a (A vs G) | d | AA vs G-G | G-A vs G-G |
| DG      | -2.03 | 0.33 | 2.31 | 0.38 | 18.59 | 0.81 | -6.45 | 0.48 | 0.82 | 0.53 | 34.04 | 0.95 |
| WW      | -0.05 | 0.47 | -0.28 | 0.95 | -0.11 | 0.70 | -0.06 | 0.50 | -0.13 | 0.73 | -0.15 | 0.76 |
| SM      | -0.10 | 0.69 | -0.10 | 0.65 | -0.19 | 0.69 | 0.02 | 0.40 | -0.13 | 0.76 | -0.12 | 0.71 |
| LW      | 0.00 | 0.32 | -0.18 | 0.88 | -0.03 | 0.44 | 0.04 | 0.49 | -0.04 | 0.48 | -0.11 | 0.73 |
| LT      | 0.02 | 0.38 | -0.21 | 0.92 | -0.07 | 0.63 | -0.06 | 0.54 | -0.04 | 0.46 | -0.14 | 0.79 |
| TM      | -0.11 | 0.60 | -0.19 | 0.74 | -0.02 | 0.35 | 0.14 | 0.65 | -0.15 | 0.70 | -0.13 | 0.64 |
| TP      | -0.09 | 0.54 | -0.22 | 0.80 | -0.04 | 0.37 | 0.06 | 0.46 | -0.14 | 0.68 | -0.10 | 0.56 |
| BT      | -0.14 | 0.82 | 0.10 | 0.67 | -0.30 | 0.99 | -0.16 | 0.80 | -0.24 | 0.97 | -0.26 | 0.97 |

DG, daily gain; WW, withers width; SM, shoulder muscularity; LW, loin width; LT, loin thickness; TM, thigh muscularity; TP, thigh profile; BT, bone thickness.

### Table 4.

| Trait   | LEP-1 | LEP-2 | LEP-3 |
|---------|-------|-------|-------|
|         | a (C vs T) | d | a (C vs T) | d | a (C vs T) | d |
| DG      | -1.53 | 0.67 | -5.19 | 0.44 | -24.84 | 0.66 | 19.93 | 0.61 | 32.00 | 0.80 | 34.53 | 0.79 |
| WW      | -0.12 | 0.73 | -0.26 | 0.93 | 0.02 | 0.46 | 0.31 | 0.75 | -0.23 | 0.78 | -0.03 | 0.46 |
| SM      | -0.15 | 0.82 | -0.31 | 0.97 | -0.01 | 0.45 | 0.24 | 0.70 | -0.20 | 0.75 | -0.03 | 0.46 |
| LW      | -0.05 | 0.51 | -0.17 | 0.86 | -0.19 | 0.69 | 0.36 | 0.84 | 0.00 | 0.42 | 0.05 | 0.52 |
| LT      | -0.09 | 0.71 | -0.24 | 0.94 | -0.15 | 0.64 | 0.31 | 0.80 | -0.07 | 0.56 | -0.01 | 0.44 |
| TM      | -0.09 | 0.54 | -0.41 | 0.98 | -0.21 | 0.63 | 0.33 | 0.72 | 0.09 | 0.52 | 0.06 | 0.48 |
| TP      | -0.13 | 0.67 | -0.45 | 0.98 | -0.32 | 0.74 | 0.51 | 0.86 | 0.06 | 0.48 | 0.10 | 0.53 |
| BT      | 0.03 | 0.41 | -0.20 | 0.89 | 0.35 | 0.86 | -0.04 | 0.49 | -0.21 | 0.76 | -0.03 | 0.47 |

DG, daily gain; WW, withers width; SM, shoulder muscularity; LW, loin width; LT, loin thickness; TM, thigh muscularity; TP, thigh profile; BT, bone thickness.
SNPs and haplotype effects were investigated.

Seven SNPs, which in other breeds showed associations with growth, feed efficiency, and carcass traits (Li et al., 2004; Buchanan et al., 2005; Sherman et al., 2008), in the Piemontese breed exhibited negligible effects on the investigated traits. These effects were of small magnitude (MYF5, NPY-2, NPY-3) or showed a very wide posterior distribution (GHRL, POMC, UCP2 and UCP3) and, therefore, will be not further discussed. In the GHR gene (Table 3), the A allele of GHR-2 had a general unfavourable additive effect on meat conformation traits and especially on BT, as well as relevant dominance effects, particularly on WW and LT. A large favourable additive effect, associated to the A allele, on DG and BT was observed for GHR-3. The effect on DG greatly increased when haplotypes of the two SNPs were considered: the association of the favourable A allele at GHR-3 with the slightly favourable G allele at GHR-2 raised the effect on DG to 34 g/d (nearly 0.45sA), with a probability for the effect of being relevant (greater than 0.1sA) as high as 95%, whereas it exerted a negative effect on muscularity and especially on BT.

As for the SNPs in the LEP gene (Table 4), the C allele at LEP-1 was consistently associated with negative values for all the traits, except BT; relevant dominance effects on the traits related to meat conformation were also observed. For LEP-2, results were comparable to those for LEP-1, with the C allele exerting negative additive effects on all traits with the exception of BT. The C allele at LEP-3 was associated with increased DG, with an estimated additive effect of 32.0 g/d (i.e., 0.42sA) and a probability of the effect being larger than 0.1sA of 80%.

For the analysis of the combined effects of the three SNPs in the LEP gene, only the most frequent haplotypes were considered: C-G-C (0.45), T-G-C (0.37) and C-C-T (0.14). Four additional rare haplotypes were found, with a cumulative frequency lower than 0.04. Compared to haplotype C-G-C, the haplotype containing all the favourable alleles (T-G-C) confirmed the favourable association with DG and showed positive effects, although of little magnitude on the other traits (Table 5). The haplotype combining the less favourable alleles (C-C-T) showed trivial effects on DG, but surprisingly positively affected meat conformation traits, particularly those related to the muscularity of the fore part of the body (WW and SM).

A large positive effect of the C allele at IGF2 was detected for DG (24.14 g/d), whereas small negative additive effects were observed for meat conformation traits (Table 6). For LEPR (Table 6), a relevant additive effect was observed on DG, with the C allele associated to higher values (about 45 g/d); negative additive and dominance effects were observed for all the conformation traits.

### Discussion

In the past decades an increasing number of associations between SNPs in candidate genes and several production traits have been reported in beef cattle, but very often no studies were performed to validate the results, or inconsistencies were observed across populations, so that the possibility to exploit the detected associations in selection programmes was limited. The present study revealed absence of polymorphism at GDF8-1, GDF8-2, GH, GHR-1, and NPY-1 in the examined sample, and negligible effects of the SNPs in GHRL, MYF5, NPY, POMC, UCP2 and UCP3 genes. Therefore, it can be concluded that these SNPs are not suitable as markers in the Piemontese breed for the traits recorded during the performance testing. More promising results have been obtained for the remaining SNPs. The GHR is one of the most investigated genes for relationships with growth, because evidences other than its physiological role in the expression of the trait suggest it as primary candidate for traits related to growth and meat production in many species (Blair and Savage, 2002; Tixier-Boichard, 2002; List et al., 2011). Previous studies of GHR gene in cattle mainly focused on two polymorphisms in exon 10 which induce amino acid substitutions, but did not reveal any significant effect on growth traits in Angus cattle (Ge et al. 2003) nor in the

#### Table 5. Estimates of the LEP haplotype effects and marginal posterior probability (P) of the estimate of being larger than 0.1 sA.

| Trait | LEP-1 – LEP-2 – LEP-3 |
|-------|-----------------------|
|       | T-G-C vs C-G-C        | C-C-T vs C-G-C |
| Mean P Mean P | Mean P Mean P |
| DG  16.21 0.73 | 4.09 0.43 |
| WW  0.12 0.70 | 0.31 0.94 |
| SM  0.14 0.79 | 0.30 0.93 |
| LW  0.05 0.54 | 0.16 0.78 |
| LT  0.07 0.63 | 0.18 0.81 |
| TM  0.03 0.35 | 0.07 0.49 |
| TP  0.05 0.43 | 0.12 0.59 |
| BT  0.03 0.42 | 0.36 0.98 |

DG, daily gain; WW, withers width; SM, shoulder muscularity; LW, loin width; LT, loin thickness; TM, thigh muscularity; TP, thigh profile; BT, bone thickness.

#### Table 6. Estimates of additive (a) and dominance (d) effects of the single nucleotide polymorphisms in the IGF2 and LEPR genes and marginal posterior probability (P) of the estimate of being larger than 0.1 sA.

| Trait | IGF2 | LEPR |
|-------|------|------|
| a (C vs T) | d | a (C vs T) | d |
| Mean P Mean P | Mean P Mean P |
| DG  24.14 0.86 | 15.59 0.67 | 44.80 0.74 | 3.42 0.47 |
| WW  -0.06 0.52 | -0.34 0.96 | -0.35 0.75 | -0.50 0.82 |
| SM  -0.03 0.41 | -0.22 0.86 | -0.11 0.55 | -0.39 0.76 |
| LW  -0.02 0.40 | -0.15 0.78 | -0.13 0.59 | -0.39 0.79 |
| LT  -0.09 0.66 | -0.22 0.90 | -0.29 0.75 | -0.69 0.95 |
| TM  -0.08 0.52 | -0.10 0.56 | -0.42 0.76 | -0.81 0.92 |
| TP  0.02 0.35 | -0.03 0.40 | -0.43 0.77 | -0.88 0.94 |
| BT  -0.06 0.53 | -0.09 0.62 | -0.34 0.76 | -0.34 0.76 |

DG, daily gain; WW, withers width; SM, shoulder muscularity; LW, loin width; LT, loin thickness; TM, thigh muscularity; TP, thigh profile; BT, bone thickness.
Piemontese breed (Di Stasio et al., 2005), leading to the conclusion that GHR gene did not seem a useful marker for traits related to growth.

On the contrary, two of the SNPs here investigated (GHR-2 and GHR-3) showed relevant associations with daily gain, specially when the haplotypes at the two SNPs were considered. In addition, when the examined sample was subdivided into two groups, one including the individuals selected for artificial insemination and the other the culled candidates, on the basis of the selection index of the Piemontese breed which includes daily gain with a weight of 14%, a significantly higher frequency of the favourable G allele at GHR-2 in the selected group was observed (0.63 vs 0.51; P=0.01). As changes in allele frequencies of a SNP in the direction expected because of the selection could contribute to validate a putative marker (Ron and Weller, 2007), the finding provides further evidence that these SNPs at the GHR gene affect daily gain. The favourable effect of the A allele at GHR-3 on daily gain was previously observed by Sherman et al. (2008) in experimental animals of composite breeds, even if, in opposition to our results, the effects were reduced when haplotypes were considered.

Together with the genes of the somatotropic axis, the LEP gene is one of the most intensively studied for relationships with feed intake and fat-related traits in cattle, whereas fewer data exist on its effects on growth (Nkrumah et al., 2005; Di Stasio et al., 2007). Associations of the TT genotype at LEP-1 with increased leptin concentration, backfat thickness and marbling score, as well as with greater feed intake, growth rate and live weight at slaughter were reported in crossbred animals (Nkrumah et al., 2005). The increased daily gain associated to the T allele was confirmed by the present data. As during the performance testing the animals were fed the same diet under restricted conditions, the association with growth indirectly suggests an improved feed conversion, in agreement with Crews et al. (2004) and Nkrumah et al. (2005). This could have a relevant practical impact because improvement in feed efficiency could contribute to reduce the feed costs, thus increasing the profitability of beef production. A greater frequency of the favourable T allele (0.42) was observed in Piemontese animals relative to the frequency reported for other populations (Nkrumah et al., 2005; Schenkkel et al., 2005). The favourable effects of the G allele at LEP-2 on most traits was not unexpected, considering the marked linkage disequilibrium with LEP-1, previously detected in other breeds also (Nkrumah et al., 2005; Schenkkel et al., 2005). The associations found are in agreement with those described by Nkrumah et al. (2005), who reported increased feed intake, growth rate and body weight associated to GG genotype at this SNP.

As for LEP-3, the results of previous investigations on the relationships with meat production traits were rather inconsistent, showing either association with carcass fatness (Buchanan et al., 2002; Lim et al., 2004; Schenkel et al., 2005), or no effect on feed intake and fatness traits (Lagonigro et al., 2003; Barendse et al., 2005). The present data revealed a highly favourable effect of the C allele at LEP-3 on daily gain, consistently with results obtained in another hyperbrophic breed, the Blonde d’Aquitaine, where the C allele positively affected daily gain, with a large and significant effect corresponding to 0.66 phenotypic standard deviation (Di Stasio et al., 2007). Other studies showed that the T allele was associated with increased milk production (Buchanan et al., 2003), whereas crossbred CT and TT cows were reported to wear heavier calves (DeVuyst et al., 2008).

Insulin-like growth factors belong to the class of polypeptides involved in the regulation of cell development, and therefore the coding genes have been proposed as candidates for growth and production in livestock. One of these genes, IGF2, is imprinted in cattle (Dindot et al., 2004), as in other mammalian species, but undergoes a postnatal loss of imprinting (Goodall and Schmutz, 2007), so that only the paternal allele is expressed during the foetal life, while both alleles are expressed after birth. Recently, imprinted genes, including IGF2, were confirmed as candidates for beef production traits in Limousin breed, supporting their role in animal growth and development (Magee et al., 2010). Associations of the IGF2 polymorphism here considered with birth weight were reported in different beef populations and crossbreds, and selection for CC sires was proposed to ensure lower birth weight in order to reduce dystocia risks (Schmutz and Goodall, 2005; Goodall and Schmutz, 2007). The same Authors also found that CC animals had larger rib-eye area, which affects the economic return of the carcass. Effects on body weight, daily gain, feed conversion and rib eye area were also detected by Sherman et al. (2008), but for rib eye area they were in the opposite direction compared to findings of Goodall and Schmutz (2007). Our results also revealed associations of IGF2 with growth, but indicated a positive effect of the C allele on daily gain, and indirectly on feed efficiency for the reasons previously mentioned, which is opposite to the results of Sherman et al. (2008), who found that TT animals had a greater daily gain and lower feed conversion ratio. Few studies exist on LEPR gene in cattle. The SNP here considered was shown to be associated with leptin concentration during late pregnancy in Friesian breed (Liefers et al., 2004), while no relationships with daily gain were found in Aberdeen Angus and Charolais breeds (Almeida et al., 2008). In opposition to the findings in beef cattle, the present study revealed that the LEPR had the largest effect on daily gain. This result deserves further investigations, for the impact it can have for the genetic improvement of the breed.

Conclusions

The study investigated the variability of twelve candidate genes in the Piemontese breed, showing relevant associations of SNPs in GHR, LEP, IGF2 and LEPR genes with traits recorded during the performance testing of Piemontese bulls. Although further studies would be useful to confirm the results for IGF2 and LEPR, the associations observed for GHR and LEPR were consistent with those of previous studies, providing additional evidence of their usefulness as markers.

Incorporating information of these markers in the breeding programme of the Piemontese cattle might increase the rate of genetic gain for some of the traits in the breeding goal of the population. Of course, before suggesting practical use of the investigated polymorphisms, evaluation of costs, operational aspects and extra gain relative to traditional breeding programmes exploiting only polygenic effects need to be performed.

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