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To cite this article: Bruno Stefanon, Romina Floris, Silvia Braglia, Roberta Davoli, Luca Fontanesi, Stefania Dall’Olio, Giorgio Graziosi, Piero Susmel & Vincenzo Russo (2004) A new approach in association study of single nucleotide polymorphism of genes for carcass and meat quality traits in commercial pigs, Italian Journal of Animal Science, 3:2, 177-189, DOI: 10.4081/ijas.2004.177

To link to this article: https://doi.org/10.4081/ijas.2004.177
A new approach in association study of single nucleotide polymorphism of genes for carcass and meat quality traits in commercial pigs

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Paper received October 20, 2003; accepted February 2, 2004

ABSTRACT

Six batches of four commercial hybrids of heavy pigs, reared for the production of Italian dry-cured hams, were identified for having homogeneous feeding and farm conditions. For a total of 235 pigs, slaughtered in the same slaughterhouse, carcass traits and muscle composition were measured. The pigs were genotyped for single nucleotide polymorphisms (SNPs) of Na+, K+-ATPase subunit alpha 2 (ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide; ATP1A2), cystatin B (CSTB), mitochondrial 2,4-dienoyl-CoA reductase 1 (DECR1), leptin (LEP; 3 SNPs), melanocortin receptor 4 (MC4R), melanocortin receptor 5 (MC5R), sarcolipin (SLN) and titin (TTN) genes. All genes showed biallelic polymorphisms and the alleles were differently distributed between the six batches. Pigs were subsequently clustered in "lean" and "fat" using either carcass traits (lean percentage, backfat thickness, loin muscle thickness, ham weight and ham cover fat thickness: 100 lean and 135 fat) or meat composition data (dry matter, protein, fat and ash of Biceps femoris and Vastus lateralis and pH after 24 hours: 126 lean and 109 fat). The association of gene polymorphisms with leaness and fatness of pigs was thus investigated using a logistic regression. ATP1A2, LEP (HinfI polymorphism) and MC4R, together with sex and ham weight were included in the model to screen lean and fat pigs classified according to carcass traits data, yielding a correct classification of 71%. For the lean and fat pigs classified according to muscle composition, sex, CSTB, DECR1, MC5R and LEP (AciI/TaqI polymorphisms) were included in the regression analysis, that yielded a 66% of pigs correctly classified.

These preliminary results may indicate that some of the selected candidate genes could be associated to production traits and are worth of further investigations.

Key words: Meat quality, Carcass composition, Gene polymorphisms, San Daniele ham, Pigs

RIASSUNTO

STUDIO DEI POLIMORFISMI DA MUTAZIONI PUNTIFORMI DI GENI CANDIDATI PER IL CONTROLLO DELLA DEPOSIZIONE DI GRASSO E LA PRODUZIONE DI CARNE IN SUINI COMMERCIALI

Per questo studio sono stati utilizzati 235 ibridi di suini pesanti, selezionati per la produzione di prosciutti di San Daniele secondo il regolamento dell’omonimo Consorzio. Gli animali, appartenenti a quattro tipi genetici, provenivano da sei
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Introduction

The assessment of economic value of carcass and meat quality traits is relevant for genetic companies, producers, processors, retailers and food service sector.

The genetic improvement of meat quality for the production of dry-cured hams, like Parma and San Daniele, is the main purpose of the Italian pig breeding industry and some commercial lines have been obtained for this aim. The final quality of the meat is largely influenced by several parameters of the meat: loin muscle thickness, backfat thickness and intermuscular fat, fat composition, proteinases activity, meat percentage and ham weight (Russo and Nanni Costa, 1995). In Italy a few studies have been carried out to estimate the heritability of these traits (i.e.: Buttazzoni et al., 1993; Russo et al., 2000) that can be evaluated only post-mortem. The DNA markers associated to these traits could have an important role to improve meat quality and to exploit new strategies in marker assisted selection programs.

The porcine genetic maps developed during the last years (Archibald et al., 1995; Ellegren et al., 1994; Marklund et al., 1996; Rohrer et al., 1994; 1996) represent important tools to map quantitative traits loci (QTLs) for meat and quality production traits using genome scanning approaches (Andersson-Eklund et al., 1998; de Koning et al., 2001; Malek et al., 2001; Milan et al., 2000; Ovilo et al., 2002; Paszek et al., 2001; Quintanilla et al., 2002; Rohrer et al., 1998).

Moreover, several studies use a candidate gene approach to identify genes affecting economic traits (i.e.: Gerbens et al., 1998; Drogemuller et al., 2001; Casas-Carrillo et al., 1997; Grindflek et al., 2002).

Some of these investigated genes are involved in the fat and meat deposition and growth regulation processes. Jiang and Gibson (1999) studied the variation in the porcine leptin (LEP; known also as obese, ob) gene and identified that allele frequencies of some single nucleotide polymorphisms (SNPs) differed significantly in groups of pigs selected for divergent backfat thickness. Moreover, two polymorphisms at this locus may be associated with feed intake and growth rate in Landrace pigs (Kennes et al., 2001). Kim and Larsen. (2000) showed that a missense mutation in the melanocortin receptor 4 (MC4R) gene affected growth and carcass traits in Large White, Landrace, Duroc and commercial pig lines selected...
for the production of light pigs. Furthermore, Russo et al. (1999) identified a SNP in the ATPase subunit alpha 2 gene (ATP1A2), localized on porcine chromosome (Sscr) 4 in a region in which several research groups have mapped QTLs for fat deposition, carcass traits and average daily gain (Andersson et al., 1994; Bidanel et al., 2001; de Koning et al., 1999; Grindflek et al., 2001; Pérez-Enciso et al., 2000, Walling et al., 1998; 2000; Wang et al., 1998, Wimmers et al., 2002). Preliminary results obtained using this SNP indicated a putative effect of the ATP1A2 locus with meat production and fatness (Russo et al., 1999; 2000).

In the present study we considered the ATP1A2, LEP and MC4R genes for which some effects on production traits have been already reported. Moreover we selected other five candidate genes on the basis of their chromosomal localisation in QTL regions and the function of the coded protein on energy intake (melanocortin receptor 5: MC5R), subcutaneous fat (mitochondrial 2,4-dienoyl-CoA reductase 1: DECR1) and muscle deposition (cystatin B: CSTB; sarcolipin: SLN; titin: TTN) as reported in Table 1. Markers of all these eight candidate genes were tested in association studies with meat production traits in commercial lines of pigs reared for the production of San Daniele ham using the logistic regression approach.

Material and methods

Animals, housing and slaughtering

Six batches were used. Pigs were bred in six farms trying to make breeding conditions as similar as possible.

Four batches were commercial hybrids: two from SCAPAAG (H1 and H4), one from JSR (H5) and one from PIC (H6); two batches were cross-breed of Large White x Landrace (H2 and H3). For each batch, 60 contemporary piglets (30 females and 30 castrate males) were initially selected in commercial farms, according to the rules of the Consortium of Prosciutto di San Daniele. Since not all the animals reached the final slaughter weight (about 160 kg live weight) within the same fattening period, the number of pigs reaching the recommended final live weights for each batch was lower than the initial, as reported in Table 2.

Initial total live weight for each batch was recorded and the plane of nutrition was programmed to ensure that the same commercial feeds, administered in similar amount between the six batches, would be used. Animals were housed in batches, not divided by gender, and grown until slaughter with three commercial feeds according to the following phases: phase-1, ad libitum, from the beginning to 60 days; phase-2, ad libitum from 61 to 120 days, and phase-3, restricted from 121 days until the end (208 days on average). The three phases corresponded to the administration of different commercial concentrate feeds. The chemical composition of the concentrates fed used during the 3 phases to six batches of pigs was: crude protein 16.2, 15.4 and 14.2%; lipids 5.2, 5.2, and 4.9%; crude fibre 5.6, 6.2, 5.0%; ash 7.2, 6.9 and 6.2%; lysine 0.91, 0.83 and 0.72%.

For the six batches, initial and final live weights, average daily gain, length of rearing and total feed intakes and efficiency are reported in Table 2.

On the day of slaughtering, all pigs were delivered to the commercial slaughterhouse where they rested for a minimum of 12 hours prior to slaughtering. Pigs were then selected to have a hot carcass weight within the range of 125 and 140 kg, and the weight was recorded. Fat and loin muscle thickness (backfat thickness and loin thickness) between the third and fourth last rib on the left hot carcass were measured using a Fat-O-Meat’er instrumentation, then carcass lean percentage was calculated and hot carcass weight was recorded for each selected pig. After jointing and fat thickness recording, the right ham of each pig was weighed. Subsequently, the hams were trimmed and cured according to the production regulations of the Consortium of San Daniele ham. Samples (about 80 g) of Biceps femoris and Vastus lateralis muscles from each pig were collected and intermuscular fat was manually removed. The muscle samples were stored at -30°C for subsequent analysis. For each sample, intramuscular fat content was determined using Soxhlet petroleum-ether extraction, crude protein using Kjeldahl analysis, ash (550°C for 12 h) and dry matter (105°C for 48 h).

Moreover, a further muscle sample of Biceps femoris was taken for DNA extraction. After 24 hours from slaughtering, pHu was measured in
Table 1. List of the investigated genes with a description of the protein product functions. PCR primers and conditions are reported as well as map position and method of analysis of the SNPs.

| Gene symbol | Gene name | Function of the coded protein | Primer sequence | PCR conditions | Chromosomal localization | Method of analysis | SNP | Reference |
|-------------|-----------|--------------------------------|-----------------|---------------|--------------------------|------------------|-----|-----------|
| ATP1A2      | Na+, K+-ATPase transporting alpha 2 polypeptide | enzyme responsible for the ATP-dependent transport of Na+ and K+ ions across the plasma cell membrane | For: 5'-ACCCTAGGAAATGGAAGAC-3' <br> Rev: 5'-TGGGCTCAAACTTCACAGTG-3' | 219/57/1.5/R/P | 4q15-q16 | SSCP | G/C | Fontanesi et al., 1999; Russo et al., 1999 |
| CSTB        | Cystatin B | small protein belonging to the cysteain proteinase inhibitor superfamily | For: 5'-GAAGGCTGGGCGTGTCATC-3' <br> Rev: 5'-GGTCAAGGGCTGGTCTCAG-3' | 229/57/1.5/R/P | 13>1/2q41 | PCR-RFLP | A/G | Russo et al., 2002 |
| DECR1       | Mitochondrial 2,4-dienoyl-CoA reductase 1 | involved in the beta oxidation of polyunsaturated enoi-CoA | For: 5'-CAGCTAGTCTCCCTGAGAC-3' <br> Rev: 5'-GGAAGGAAGATGGAGAGGCAC-3' | 133/51/1.0/R/M | 4q15-q16 | PCR-RFLP | G/C | Davoli et al., 2002 |
| MC4R        | Melanocortin receptor 4 | its inactivation is related to obesity in man and mouse | For: 5'-TACCCTGACATCTGTGAG-3' <br> Rev: 5'-GCCTGTTGATTCTTATG-3' | 226/58/1.5/R/M | 1q22-q27 | PCR-RFLP | G/A | Kim et al., 2000a |
| MC5R        | Melanocortin receptor 5 | responds to melanocortin by increasing intracellular cAMP and thermogenesis | For: 5'-TCAGCCTGCTGAGAGACATC-3' <br> Rev: 5'-GCCACCAAGGAGACAGAGC-3' | 237/58/1.5/R/M | 6q24-(1/2)q31 | PCR-RFLP | A/G | Kim et al., 2000b |
| LEP         | Leptin | considered a relevant central anorexogenic protein | For: 5'-GAAGCAGATCTTCCTGAGAG-3' <br> Rev: 5'-AGACCTCGAGGAGGACAG-3' | 469/58/1.5/R/M | 18q13-q21 | PCR-RFLP | C/T | Jiang and Gibson, 1999 |
| LEP         | Leptin | considered a relevant central anorexogenic protein | For: 5'-CACTCACTCGCTGTTCTGAT-3' <br> Rev: 5'-AGGGAAAGGAGGAGGAGGAG-3' | 569/58/1.5/R/M | 18q13-q21 | PCR-RFLP | A/G | Jiang and Gibson, 1999 |
| LEP         | Leptin | considered a relevant central anorexogenic protein | For: 5'-GTATCGAGGAGGAGGAGGAC-3' <br> Rev: 5'-ACAGGATCTTCCTGAGAGGAG-3' | 264/58/1.5/R/M | 18q13-q21 | PCR-RFLP | C/G | Sawaki et al., 1996 |
| SLN         | Sarcolipin | regulates the activity of a fast-twitch skeletal muscle sarcoplasmic reticulum ATPase | For: 5'-ATATGGGTCTCTGAGAGGAAG-3' <br> Rev: 5'-CTTGGGAGATGACAGATGGTGA-3' | 218/57/1.0/R/M | 9p24-(1/3)p21 | PCR-RFLP | A/G | Fontanesi et al., 2001 |
| TTN         | Titin | involved in muscle assembly and ultrastructure, the largest known protein | For: 5'-MCTAATGGTGAGGAGAGA-3' <br> Rev: 5'-GTAATAGTCTTCCTTGAGGAT-3' | 195/53/2.5/G/P | 15q23-q26 | SSCP | C/T | Davoli et al., 2003 |

1. According to UniGene (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unigene).
2. Length in bp/optimal annealing temperature/[MgCl2]/Taq Polymerase: S=Sigma, G=AmpliTaq Gold (Applied Biosystems Foster City U.S.A.), R=Roche Diagnostics (Manheim Germany)/thermal cycler: P= Perkin Elmer 9600,M= MJR PT100.
Analyses of the SNP polymorphisms
DNA was extracted using a standard protocol (Sambrook et al. 1989).

Primers for the amplification of the candidate genes were designed on available genomic or cDNA sequences and are reported in Table 1 that includes also PCR conditions and amplicon length for each locus.

Single strand conformation polymorphism (SSCP) analyses, with the protocol described by Fontanesi et al. (2001), were used to genotype the markers at the ATP1A2 and TTN loci.

PCR-RFLP protocols were applied to analyse the SNPs at the CSTB, DECR1, LEP, MC4R, MC5R and SLN loci using the restriction enzymes reported in Table 1. Restriction products were visualised by agarose (3% SeaKem) or polyacrilamide/bisacrylamide 29:1 gel electrophoreses.

Statistical analysis
Statistical analysis were performed with the 7.5 version of the SPSS package (1997).

First of all, the GLM procedure was used to evaluate the part of variance of the analysed traits due to batch, sex and their interaction as fixed effect in the model and to estimate differences in carcass traits and muscle composition among the six batches for the same traits.

To separate fat from lean animals, in a second analysis, pigs of the six batches were pooled and split in “lean” and “fat” on the basis of carcass traits (ham cover fat, backfat and loin thickness and lean meat percentage) or muscle composition (dry matter, ash, protein and lipid contents and pHu of Biceps femoris). This classification was realised using the “cluster k-mean” procedure of the SPSS package, release 7.0 (SPSS, 1997), with a k value of 2. The efficiency of the clustering, thus the verification of the differences between “lean” and “fat” pigs, was assessed by mean of a comparison procedure (ANOVA). The analysis of variance compared the mean values of carcass and muscles variables with cluster as fixed effect 2 levels: a value of 0 (lean pigs) or 1 (fat pigs) was assigned to the derived dichotomous population, each of them including castrate and female pigs of the six batches.

To test the association of the polymorphisms of the candidate genes with carcass and meat quality traits across the batches, the newly-defined binary trait was used as dependent variable in the logistic regression, with SNPs and sex as independent categorical variables and live weight as quantitative covariate, using a stepwise procedure with the default probability of input (0.15) of the SPSS package (1997). Since neither homoscedasticity nor normally distributed variables are required for binary logistic regression, data were not pre-processed and were directly used in the statistic computation.

The approach was applied to predict a dependent dichotomous variable on the basis of inde-

| Commercial hybrid | Batch | Female | Male | Total | Live weight, kg mean±SD | Daily gain, g/d mean±SD | Length, kg feed kg weight | Length, kg weight |
|-------------------|-------|--------|------|-------|-------------------------|--------------------------|--------------------------|-------------------|
| SCAAPAG H1        | 16    | 17     | 33   | 25.8+0.25 160.3+6.28 | 669+31 201 3.45 | |
| LwxL H2           | 17    | 19     | 36   | 25.2+0.55 157.2+8.29 | 603+38 219 3.78 | |
| LwxL H3           | 23    | 28     | 51   | 26.3+0.24 155.9+8.91 | 617+42 210 3.67 | |
| SCAAPAG H4        | 23    | 24     | 47   | 28.1+0.21 166.0+7.78 | 638+36 216 3.66 | |
| JSR H5            | 13    | 22     | 35   | 29.0+0.21 155.6+6.11 | 639+31 198 3.87 | |
| PIC H6            | 15    | 18     | 33   | 25.6+0.18 158.1+3.64 | 663+18 200 3.40 | |
| Mean              |       |        |      | 26.7±1.40 158.9±8.29 | 638.2±41 207.3 3.64 | |
dependent and to rank their relative importance, giving an estimate of the probability that a certain event occurs (Rice, 1994). The approach was preferred to the general linear model because it allows to include all loci as independent variables in the same analysis, instead of evaluate the effect of each locus on carcass traits.

The success of the logistic regression was evaluated in terms of classification table (reporting correct and incorrect classification of individuals for the dichotomous variable), χ² goodness-of-fit test (model χ²) and Wald statistic (to test the significance of individual independent variables).

**Results and discussion**

The comparison of the recorded data of the 235 pigs used in the present research with those used as reference for the regulation of the production of San Daniele ham showed that average initial and final live weights, daily gain and feed efficiency for the six batches of pigs were within the accepted ranges for commercial farms in Italy (Lo Fiego et al., 2000; Martelli et al., 2002). Furthermore, the green hams of the 235 pigs used for the present research were considered suitable for the production of San Daniele ham according to the regulations approved by the San Daniele official board.

The results of ANOVA showed a significant effect of sex for almost all the carcass traits and muscle composition, with the exception of carcass weight, pHu and protein content of Vastus lateralis (Table 3).

| Carcass traits: | Probability of the main sources of variation |
|----------------|---------------------------------------------|
| Weight kg      | Batch (B) 0.000 0.069 0.256 32.897 0.298   |
| Lean meat %    | - 0.000 0.000 0.431 12.649 0.329           |
| Backfat thickness mm | 0.000 0.000 0.000 0.791 16.431 0.354    |
| Loin thickness " | 0.197 0.000 0.000 0.373 38.280 0.235    |
| Ham weight kg   | 0.000 0.000 0.032 0.367 0.833 0.366    |
| Ham fat thickness mm | 0.002 0.000 0.002 0.015 21.372 0.386  |

**Biceps femoris (BF):**

| Dry matter % | 0.052 0.000 0.049 0.098 0.460 0.321 |
| Protein " | 0.391 0.000 0.068 0.065 0.456 0.344 |
| Fat " | 0.396 0.000 0.004 0.436 0.792 0.143 |
| Ash " | 0.451 0.000 0.008 0.345 0.000 0.274 |
| pHu BF | 0.879 0.000 0.651 0.019 0.014 0.488 |

**Vastus lateralis (VL):**

| Dry matter % | 0.301 0.000 0.000 0.923 0.738 0.238 |
| Protein " | 0.345 0.000 0.024 0.372 0.581 0.224 |
| Fat " | 0.730 0.000 0.000 0.600 0.905 0.212 |
| Ash " | 0.688 0.000 0.001 0.423 0.000 0.167 |
Table 4. Least square means (LSM) and standard errors (SE) of carcass traits and muscle compositions for the six batches of pigs.

| Batch | LSM  | SE  | LSM  | SE  | LSM  | SE  | LSM  | SE  | LSM  | SE  | LSM  | SE  |
|-------|------|-----|------|-----|------|-----|------|-----|------|-----|------|-----|
| Carcass traits: |      |     |      |     |      |     |      |     |      |     |      |     |
| Weight kg | 133.82 | 0.80 B | 132.55 | 1.13 B | 127.56 | 1.07 C | 139.02 | 0.87 A | 131.49 | 0.71 B | 130.92 | 0.45 BC |
| Lean meat % | 48.31 | 0.69 BC | 44.02 | 0.80 D | 46.87 | 0.24 C | 52.01 | 0.51 A | 49.47 | 0.76 B | 48.60 | 0.66 BC |
| Backfat thickness mm | 24.35 | 0.59 B | 29.85 | 0.87 A | 28.72 | 0.60 A | 20.57 | 0.53 C | 24.17 | 0.83 B | 25.52 | 0.69 B |
| Loin thickness | 53.32 | 1.60 B | 55.03 | 1.32 B | 51.92 | 0.72 B | 58.02 | 0.62 A | 58.83 | 1.03 A | 59.96 | 0.96 A |
| Ham weight kg | 14.24 | 0.25 A | 13.04 | 0.20 C | 13.28 | 0.10 C | 14.04 | 0.10 AB | 13.14 | 0.13 C | 13.47 | 0.17 BC |
| Ham fat thickness mm | 26.79 | 0.59 B | 31.33 | 0.79 A | 33.22 | 0.77 A | 22.69 | 0.57 C | 27.01 | 1.08 B | 24.83 | 0.83 BC |
| Biceps femoris (BF): |      |     |      |     |      |     |      |     |      |     |      |     |
| Dry matter % | 26.06 | 0.12 A | 25.92 | 0.08 A | 24.88 | 0.10 B | 25.43 | 0.12 BC | 25.89 | 0.13 A | 25.53 | 0.10 C |
| Protein % | 22.26 | 0.12 B | 22.97 | 0.11 A | 21.85 | 0.08 C | 21.95 | 0.12 C | 23.07 | 0.14 A | 22.39 | 0.11 B |
| Fat % | 2.65 | 0.17 A | 1.77 | 0.11 C | 1.85 | 0.11 BC | 2.31 | 0.15 AB | 1.64 | 0.16 C | 1.97 | 0.17 BC |
| Ash % | 1.15 | 0.00 D | 1.17 | 0.00 B | 1.17 | 0.00 B | 1.16 | 0.00 C | 1.18 | 0.00 B | 1.17 | 0.00 A |
| pHu BF | 5.41 | 0.01 D | 5.60 | 0.02 C | 5.72 | 0.02 AB | 5.68 | 0.02 B | 5.78 | 0.02 A | 5.74 | 0.02 AB |
| Vastus lateralis (VL): |      |     |      |     |      |     |      |     |      |     |      |     |
| Dry matter % | 25.57 | 0.19 B | 26.85 | 0.13 A | 25.69 | 0.12 B | 25.60 | 0.11 B | 25.56 | 0.16 B | 25.47 | 0.14 B |
| Protein % | 20.99 | 0.19 C | 22.16 | 0.12 A | 21.00 | 0.10 C | 21.63 | 0.10 B | 21.70 | 0.13 AB | 21.73 | 0.11 AB |
| Fat % | 3.50 | 0.19 A | 3.50 | 0.16 A | 3.55 | 0.13 A | 2.82 | 0.14 B | 2.69 | 0.21 B | 2.57 | 0.14 B |
| Ash % | 1.15 | 0.01 BC | 1.16 | 0.00 AB | 1.14 | 0.00 C | 1.16 | 0.00 AB | 1.17 | 0.00 A | 1.16 | 0.00 AB |

A,B,C: LSM with different superscripts on the same row statistically differ for P<0.01.
although statistically relevant, and were in the same direction of carcass composition, H5 pigs showing the leanest hams (both for Biceps femoris and Vastus lateralis).

The biallelic polymorphism for each gene analysed in the present research was already described in literature (Davoli et al., 2002; 2003; Floris et al., 2001; Fontanesi et al., 1999; 2001; Jiang and Gibson, 1999; Kim and Larsen, 2000; Russo et al., 2002). In the analysed population all the genes were polymorphic and both allelic variants were detected. Moreover, 3 genotypes were found for 6 genes (ATP1A2, DECR1, MC4R, MC5R, SLN and TTN and LEP HinfI), while no homozygous 1/1 and 2/2 individuals were observed respectively for CSTB and LEPAciI/TaqI.

The allele 2 of CSTB gene was the most frequent in agreement with the results obtained by Russo et al. (2002), who analysed the polymorphism distribution of this locus in the Italian Large White, Italian Landrace, Duroc, Piétrain, Belgian Landrace and Hampshire breeds.

The allele distribution for ATP1A2 gene was quite similar in the different batches with the exception of H5 (allele 1 = 0.23). The genotyping of DECR1 gene showed that for batches H3, H4 and H6 allele 2 was the most frequent. In batches H1, H2 and H5 the allelic distributions were near to 0.50 and these results were in agreement with those described by Davoli et al. (2002) analysing pure breeds of pigs: frequencies of allele 2 ranged from 0.40 (Landrace) to 0.63 (Large White).

Allele 1 of TTN gene was the most frequent in all batches. Moreover, batch H6 showed significant differences in the distribution of the values of allele frequencies in comparison to the other five batches.

For LEP, allele frequencies of the HinfI RFLP indicated a prevalence of allele 1 in all the 6 batches of pigs, with values ranging from 0.76 (H1, H4 and H5) to 0.91 (H6). Similar results were obtained by Jiang and Gibson (1999) in different breeds and synthetic lines of pigs, even if the vari-

| Carcass traits | Cluster 1 Lean | Cluster 2 Fat | MSE | F value | P of F |
|----------------|----------------|---------------|-----|---------|--------|
| Lean milk %    | 50.84          | 44.62         | 9.40| 236.492 | 0.000  |
| Backfat thickness mm | 22.63 | 29.68 | 13.28 | 214.742 | 0.000  |
| Loin thickness | 59.85          | 50.45         | 28.49| 177.947 | 0.000  |
| Ham fat thickness | 24.82 | 32.23 | 21.44| 146.809 | 0.000  |

Table 5. Classification of pig population in "lean" and "fat" using either carcass traits or muscle composition and pHu as independent variables in the cluster analysis k-means.
SNPs and carcass traits in heavy pigs

A complete correspondence of SNPs generated by TaqI and AciI restriction enzymes was observed in all the pigs. However, Sasaki et al. (1996) reported a lack of association in other breeds, probably indicating a linkage disequilibrium between these two SNPs.

The SNP distribution in different pig breeds and synthetic populations for MC4R was investigated by Kim and Larsen (2000), who observed a frequency of allele 1 ranging from 1.00 in Meishan pigs, down to 0.00 in Hampshire pigs, with intermediate values for other breeds (Duroc, 0.56; Chester White, 0.2). Significant differences were observed between batches and, unexpectedly, H1 and H4 (i.e. the SCAAPAG lines) had respectively the highest (0.77) and the lowest (0.22) allele 1 frequency.

For MC5R limited information is available. Kim et al. (2000) reported that the A/G substitution can vary from 0.07 in Landrace to 0.75 in Duroc, and 1.00 in Hampshire, Chester White and Yorkshire breeds. Higher allele 1 frequencies were observed for H5 and H6.

However, published data for some of the analysed genes were obtained in a limited number of pigs and, at the best of our knowledge, the results here presented give a preliminary insight of the variability of allele frequencies of DECR1, MC4R, MC5R, SLN and TTN in commercial synthetic populations.

Clustering of the six batches generated a dichotomous population, that resulted discriminated for the fatness/leaness of the carcass (i.e. carcass traits) or of the muscles (i.e. muscle composition). The subsequent analysis of variance of the newly-defined population confirmed that pigs of the "lean" group were significantly leaner than those of the "fat" group (Table 5).

The results of odds ratios from binary logistic regression are reported in Table 6. For a better understanding of the logistic regression output, an odds ratio (e^B where B is the coefficient of regression) higher than 1.00, means that the odds of being in the highest class of the dependent variable (i.e. fat) are multiplied by 1.39 when the ATP1A2 values (independent variables) increase from level 0 to level 1. Furthermore, for this SNP, homozygotes for allele 2 show an odds ratio higher than heterozygotes. However, the level of significance of Wald test (0.391) assumes the null hypothesis. These results were in agreement with Russo et al. (1999) who studying the association of ATP1A2 with carcass traits, indicated that pigs with genotype 1/1 presented a trend to have more lean meat and smaller backfat thickness than pigs with genotype 1/2 and 2/2.

The presence of allele 2 for LEP was associated to an odds ratio lower than 1, indicating a reduced fat deposition. These data are consistent with those published by Jiang and Gibson (1999), obtained in 5 commercial pig lines selected for light slaughter weight (about 100 kg). In their study, the frequency of allele 2 was significantly higher in lean compared to fat animals, although only in one batch of Large White. The authors concluded that LEP (HinfI polymorphism) is presumably associated to a QTL, but that the lack of association in other breeds could indicate a linkage disequilibrium between the putative QTL and this polymorphism. For the LEP AciI/TaqI RFLPs, a lack of association with fat deposition was reported (Sasaki et al., 1996).

According to Kim and Larsen (2000) the missense variant of the porcine MC4R is associated with fatness and obesity related traits, and these results are confirmed by the odds ratios higher than 1 reported in Table 6.

Results of logistic regression for muscle com-
position could indicate the involvement of CSTB, DECR1, MC5R and LEP AciI/TaqI RFLPs in the control of fat and protein deposition. For CSTB gene, Russo et al. (2002, 2003) found a significant association with average daily gain (ADG) since the most frequent genotype (2/2) showed a favourable effect on this trait.

Since in literature there are no studies of associations of DECR1, MC5R and LEP AciI/TaqI RFLPs with muscle composition, these genes require to be further investigated.

The success of the logistic regression was also assessed by the correct and incorrect classification of the dichotomous dependent variable (Table 7). The SNPs explained the 71 and 66 % of the variability for carcass traits and muscle composition, respectively. Moreover, for both the classifications, the model prediction was higher for lean than for fat pigs. This would mean that the impact of environmental conditions, as feeding regimes and length of rearing, is higher in the control of carcass traits, quantitative variables, than for protein accretion, that is more under genetic control.

Conclusions

The study showed that in analysed commercial lines of pigs ATP1A2, LEP (polymorphism HinfI) and MC4R could be associated to carcass traits of economic relevance, while CSTB, DECR1, MC5R

### Table 6. Output of the logistic regression to predict lean and fat pigs on the basis of SNPs and sex as categorical independent variables. a) dichotomous population clustered on the basis of carcass traits, with weight of leg as covariate; b) dichotomous population clustered on the basis of muscle composition.

| Analysis of effects | Wald test P < | Odds ratios 1 vs 0 | Odds ratios 2 vs 0 |
|---------------------|--------------|--------------------|-------------------|
| a) Carcass composition: |
| ATP1A2              | 0.391        | 1.398              | 2.920             |
| LEP (HinfI)         | 0.059        | 0.472              | 0.621             |
| MC4R                | 0.085        | 2.163              | 1.313             |
| Sex                 | 0.002        | 0.386              | -                 |
| Leg weight          | 0.000        |                    | 1.876             |
| Constant            | 0.000        |                    |                   |
| Model χ²            | 0.000        |                    |                   |
| b) Muscle composition: |
| CSTB                | 0.069        |                    | 2.153a            |
| DECR1               | 0.031        | 0.562              | 0.307             |
| MC5R                | 0.056        | 2.008              | 0.012             |
| LEP (AciI/TaqI)     | 0.082        | 4.089              | -                 |
| Sex                 | 0.005        | 2.759              | -                 |
| Constant            | 0.085        |                    |                   |
| Model χ²            | 0.000        |                    |                   |

For SNP: Level 0 = homozygote for allele 1; Level 1 = heterozygote; Level 2 = homozygote for allele 2; Sex: 0 = female; 1 = male
Odd ratios: indicator contrast, initial level; a value below or above 1 indicates that a unit change in the independent variable is associated with a decrease or an increase in the odds of the dependent variable, respectively.
Model χ²: Significance of the Model log-likelihood test
a for CSTB the comparison was made between level 1 and level 2.
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and LEP (polymorphisms TaqI/AciI) could be involved in fat and protein deposition.

The approach adopted in the present research is innovative, but required to be validate with further experiments. However, it would offer the opportunity of studying simultaneously the effects of several SNPs on production traits.

We thank Martini Alimentari S.N.C., Castiglione di Ravenna, Italy, for their assistance and cooperation.

This research was supported by MURST project 2001/2002.

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