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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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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 subsequentely 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

diversi allevamenti. I soggetti sono stati allevati in condizioni uniformi di alimentazione e sono stati macellati tutti nello stesso macello. Dopo la macellazione sono stati rilevati sulla carcassa lo spessore del grasso di copertura del prosciutto, lo spessore del lardo dorsale e della lombata e la percentuale di tagli magri. Inoltre su campioni dei muscoli Biceps femoris e Vastus lateralis sono state determinate le seguenti caratteristiche di composizione: sostanza secca, ceneri, proteine, lipidi e pHu. Dal muscolo Biceps femoris è stato prelevato un ulteriore campione di tessuto utilizzato per l'estrazione del DNA. Tutti i suini sono stati genotipizzati per 10 mutazioni puntiformi (SNP) in 8 diversi geni candidati (Na^+, K^+-ATPase subunità alfa 2, ATP1A2; cistatina B, CSTB; 2,4-dienoil-CoA riduttasi mitocondriale 1, DECR1; recettore della melanocortina 4, MC4R; recettore della melanocortina 5, MC5R; leptina, LEP: 3 SNP; sarcolipina, SLN; titina, TTN) sulla base della loro localizzazione cromosomica in regioni QTL per diversi caratteri produttivi e qualitativi della carne e della carcassa e sulla base della funzione della proteina codificata. I polimorfismi biallelici analizzati nei diversi geni sono stati studiati con metodo SSCP per ATP1A2 e TTN, mentre per gli altri sei marcatori sono state utilizzate procedure di analisi PCR-RFLP. L'associazione tra i polimorfismi dei geni studiati con i caratteri della carcassa o con la composizione del muscolo è stata analizzata usando la regressione logistica. Inizialmente i suini sono stati divisi in due cluster denominati "magri" e "grassi" in base alle caratteristiche di carcassa o alla composizione del muscolo. La procedura utilizzata era in grado di spiegare il 71 e il 66 % della variabilità rispettivamente per le caratteristiche di carcassa e della composizione del muscolo.
I risultati ottenuti dall'analisi di regressione logistica suggeriscono che ATP1A2, LEP (polimorfismo HinfI) e MC4R potrebbero essere coinvolti nel controllo della composizione della carcassa, mentre CSTB, DECR1, MC5R e LEP (polimorfismi AciI/TaqI) potrebbero avere effetto sulla composizione del muscolo. L'approccio di analisi utilizzato nella ricerca è promettente e tale procedura può essere considerata uno strumento utile per studiare contemporaneamente gli effetti di più SNP su caratteristiche produttive di interesse zootecnico.

Parole chiave: *Qualità della carne, Composizione della carcassa, Polimorfismo genico, Prosciutto San Daniele, Suini.*

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 ham is largely influenced by several parameters of the green legs, like ham fat coverage, intramuscular 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 crossbreed 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 Kjeldhal 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[1]	Gene name[1]	Function of the coded protein	Primer sequence	PCR conditions[2]	Porcine chromosomal localisation	Method of analyses	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′-ACCCTAAGGAAAATGGAAGAC-3′ Rev: 5′-CACGGTCAATAAATCTCAAATG-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′ Rev: 5′-GGTCAAGGGCTTGTTCTCGTG-3′	229/57/1.5/R/P	13>1/2q41 13 q46-q49	PCR-RFLP (PvuII)	A/G	Russo *et al.*, 2002
DECR1	Mitochondrial 2,4-dienoyl-CoA reductase 1	involved in the beta oxidation of polyunsaturated enoil-CoA	For: 5′-CGTCTAAGTCCTTCCCACC-3′ Rev: 5′-GCACCTAAGCTGGACAGAT-3′	133/51/1.0/R/M	4q15-q16	PCR-RFLP (BfaI)	G/C	Davoli *et al.*, 2002
MC4R	Melanocortin receptor 4	its inactivation is related to obesity in man and mouse	For: 5′-TACCCTGACCATCTTGATTG-3′ Rev: 5′-ATAGCAACAGATGATCTCTTTG-3′	226/58/1.5/R/M	1q22-q27	PCR-RFLP (TaqI)	G/A	Kim *et al.*, 2000a
MC5R	Melanocortin receptor 5	responds to melanocortin by increasing intracellular cAMP and thermogenesis	For : 5′-TCAGCCTGCTGGAGAACATC-3′ Rev: 5′-GCCACCAAGGAGATGCAG-3′	237/58/1.5/R/M	6q24-(1/2)q31	PCR-RFLP (BsaHI)	A/G	Kim *et al.*, 2000b
LEP	Leptin	considered a relevant central anorexogenic protein	For: 5′-GAGCCAACATCTCTCTCGCTGAG-3′ Rev: 5′-AGACTCCTGGAAGCTCAGGTTTCTTC-3′	469/58/1.5/R/M	18q13-q21 (HinfI)	PCR-RFLP	C/T	Jiang and Gibson, 1999
LEP	Leptin	considered a relevant central anorexogenic protein	For: 5′-CAACTCACCGTCGCTTTCTTGAT-3′ Rev: 5′-AGGGAAGCGGAGGAGCAAAG-3′	569/58/1.5/R/M	18q13-q21	PCR-RFLP (TaqI)	A/G	Jiang and Gibson, 1999
LEP	Leptin	considered a relevant central anorexogenic protein	For: 5′-GTTACCGGAATCCAGGGTCT-3′ Rev: 5′-ACAGAGTCCCTCCTGGACAA-3′	264/58/1.5/R/M	18q13-q21	PCR-RFLP (AciI)	C/G	Sasaki *et al.*, 1996
SLN	Sarcolipin	regulates the activity of a fast-twitch skeletal muscle sarcoplasmic reticulum ATPase	For: 5′-ATATGGCTTCTCGTGAGGTC-3′ Rev: 5′-TTGAGAGCAGCATCGTTAGA-3′	218/57/1.0/R/M	9p24-(1/3p21)	PCR-RFLP (TaqI)	A/G	Fontanesi et al , 2001
TTN	Titin	involved in muscle assembly and ultrastructure, the largest known protein	For: 5′-AACTAACTGTTGGTAGGAGAA-3′ Rev: 5′-GTACACATGTCAGGTGATG-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.*

triplicate directly on the Biceps femoris muscle using a combined glass electrode (Ingold, Mettler Toledo, Switzerland).

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 anymals, 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-

Table 2. Characteristics of commercial batches of heavy pigs.

Commercial hybrid	Batch	Female 107	Male 128	Total 235	Live weight, kg mean±SD Beginning	Live weight, kg mean±SD End	Daily gain, g/d mean±SD	Length, days	kg feed /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

Table 3. ANOVA of carcass traits and muscle composition for the six batches of pigs: probability (P of F) of the main sources of variation, mean square error (MSE) and R^2.

		Probability of the main sources of variation					
		Weight	Batch (B)	Sex (S)	B x S	MSE	R^2
Carcass traits:							
Weight	kg	-	0.000	0.069	0.256	32.897	0.298
Lean meat	%	0.048	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	0.009	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

pendents 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), χ^2 goodness-of-fit test (model χ^2) 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).

All traits measured at slaughter significantly differed between batches (Table 4). Hot carcass weight was the lowest for H3 pigs and the highest for H4, but H2 and H1 had similar weights. Considerable differences were measured for lean meat percentage (from 44.02 of H2 to 52.01 of H4), backfat (from 20.57 mm for H4 to 29.85 of H2) and ham cover fat thickness (from 22.69 of H4 to 33.22 for H3). H1 showed heaviest hams while H2 had the lightest. The differences between the different batches in muscle composition were lower,

Table 4. Least square means (LSM) and standard errors (SE) of carcass traits and muscle compositions for the six batches of pigs.

		Batch											
		H1		H2		H3		H4		H5		H6	
		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-

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.

		Cluster 1 Lean	Cluster 2 Fat	MSE	F value	P of F
Carcass traits	n.	135	100			
Lean meat	%	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
Muscle composition:						
Biceps femoris (BF)	n.	126	109			
Dry matter	%	25.34	25.84	0.6177	23.544	0.000
Protein	"	22.59	22.08	0.6330	24.061	0.000
Fat	"	1.57	2.60	0.6648	92.454	0.000
Ash	"	1.18	1.16	0.0002	85.587	0.000
pHu	units	5.68	5.65	0.0272	1.748	0.000
Vastus lateralis (VL)	n.	126	109			
Dry matter	%	25.29	26.39	0.6711	104.575	0.187
Protein	"	21.70	21.24	0.6987	17.811	0.000
Fat	"	2.43	4.00	0.5300	273.931	0.000
Ash	"	1.17	1.15	0.0004	71.981	0.000

ation of allele frequencies was higher.

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 an 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 Table 6. For a better understanding of the logistic regression output, an odds ratio (eB 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 are multiplied by the odd ratio value when the value of independent variables increases of 1 unit. The level of significance of Wald test higher than 0.05 assumes the null hypothesis. According to Menard (2002), large odds ratios inflate the standard error of B, lowering the Wald statistic and leading to false negative test. This means that very large effects may lead to large standard errors and small Wald χ^2 values. In the case of a model log-likelihood test with low values (<0.01), the findings based on the Wald statistics can be ignored and an overall good model fitting can be assumed.

The results of binary logistic regression could suggest an involvement of ATP1A2, LEP (polymorphism HinfI) and MC4R SNPs in the control of carcass composition. As an example, the odds ratio of 1.39, calculated for the ATP1A2 at Level 1, 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 he 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-

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	Odds ratios	
	P <	1 vs 0	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 χ^2	0.000		
b) Muscle composition:			
CSTB	0.069	-	2.153a
DECR1	0.031	0.562	0.307
MCR5	0.056	2.008	0.012
LEP (AciI/TaqI)	0.082	4.089	-
Sex	0.005	2.759	-
Constant	0.085		
Model χ^2	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 χ^2: Significance of the Model log-likelihood test
a for CSTB the comparison was made between level 1 and level 2.

position could indicate the involvement of CSTB, DECR1, MC5R and LEP Aci1AciI/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 7. Success of the logistic regression assessed by the correct and incorrect classification of the dichotomous dependent variables.

Predicted values		Observed Values		Overall
		Fat	Lean	
Carcass traits:				
Fat	n.	56	25	
Lean	"	44	110	
Total	"	100	135	
Correctly classified	%	56	82	71
Muscle composition:				
Fat	n.	68	39	
Lean	"	41	87	
Total	"	109	126	
Correctly classified	%	62	69	66

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.

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