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Analysis of *MC4R* polymorphism in Italian Large White and Italian Duroc pigs: Association with carcass traits

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ABSTRACT

The melanocortin-4 receptor (*MC4R*) gene codes for a G protein transmembrane receptor playing an important role in energy homeostasis control. In pig a single nucleotide polymorphism c.1426G>A has been identified and associated to average daily gain, feed intake and fatness traits but a lack of agreement on the effects of the gene on carcass traits in different breeds comes out from many studies. In the present study the c.1426G>A polymorphism is analysed in two Italian pig breeds, Large White and Duroc to study the association of the *MC4R* gene with some carcass traits. The results show that the c.1426G>A polymorphism affects daily gain, feed conversion ratio and ham weight in both breeds, lean cuts in the Italian Duroc and backfat thickness in the Italian Large White. The presence of MC4R mRNA transcript in different porcine tissues was analysed.

1. Introduction

The melanocortin-4 receptor (*MC4R*) gene codes for a G protein transmembrane receptor that plays an important role in the control of energy homeostasis. In mammals, this receptor was expressed primarily in the central nervous system, in regions important in the control of food intake, body weight and energy homeostasis (Adan et al., 2006; Butler, 2006; Cooper, Jenkins, Wojakiewicz, Kattesh, & Kojima, 2011; Tao, 2010). Many researches showed the significant effects of mutations in the *MC4R* gene on obesity in mice and humans (Butler, 2006; Hughes et al., 2009). In humans, more than 150 mutations in *MC4R* gene have been found (for a review see Bromberg, Overton, Vaisse, Leibel, & Rost, 2009; Tao, 2009). Some of these reported polymorphisms were associated with dominantly inherited obesity (Bell, Walley, & Froguel, 2005) and other mutations have been identified, but their effect on food intake is unknown (Tao, 2009).

In pigs, the *MC4R* gene, physically mapped to porcine chromosome 1 (SSC1) q22–q27 (Kim, Larsen, & Rothschild, 2000), consists of approximately 1 kb of coding sequence contained within a single exon. Kim, Larsen, Short, Plastow, and Rothschild (2000) analysing the porcine *MC4R* gene described a missense mutation c.1426G>A, not found in human gene, in a region coding for the seventh transmembrane region of melanocortin receptors. In this region a motif (NPLIY) highly conserved in most of the members of the G protein-coupled receptor family occurs, and the N aminoacid originates the *A* allele. In all the melanocortin receptors the motif is different because of a substitution of N (Asn) to D (Asp) aminoacid (originated by the *G* allele) in the above motif (Kim et al., 2006).

Kim, Larsen, and Rothschild (2000) and Kim, Larsen, Short, et al. (2000), analysing different crossbred pig populations, reported that animals carrying the *G* allele showed association with less backfat thickness (BFT), slower growth rate, and lower feed intake than pigs with the *A* allele that was associated with higher fat deposition, higher feed consumption, and faster growth.

Much research has been performed in commercial pig lines as well as in experimental populations to verify the association between this SNP with fatness, daily gain, and feed intake (Bruun, Jørgensen, Nielsen, Andersson, & Fredholm, 2006; D'Andrea, Pilla, Giuffra, Waddington, & Archibald, 2008; Hernández-Sánchez, Visscher, Plastow, & Haley, 2003; Houston, Cameron, & Rance, 2004; Kim, Larsen, & Rothschild, 2000; Kim, Larsen, Short, et al., 2000; Kim et al., 2004; Meidtner et al., 2006; Piórkowska et al., 2010). The favourable effect of AA genotype on growth and backfat thickness has been reported in many papers, with some exception. For example, Park, Carlborg, Marklund, and Andersson (2002) described a positive effect of the G allele on backfat thickness and no effect on growth in the analysed populations. These authors suggest that their results, which disagree with other studies, are due to a lack of power of the study because of the small number of analysed animals or because of a very small effect of the mutation in their crossbred population.

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Moreover Stachowiak, Szydlowski, Obarzanek-Fojt, and Switonski (2006) showed the absence of a gene effect on growth, feed intake and backfat in purebred Polish pigs.

From these association studies a lack of agreement on the effects of the porcine gene on carcass traits is apparent.

Functional studies on the cellular processes influenced by the SNPs in human *MC4R* gene showed that many mutations of the gene seem to affect intracellular cAMP levels. In pigs, a functional analysis performed by Kim et al. (2004) suggested that the c.1426G>A polymorphism may have causal effects through an alteration in cAMP production by MC4R in response to ligand-binding. However Patten, Daniels, Suzuki, Fluharty, and Yee (2007), analysing artificial c.1426G>A mutation in human MC4R gene, found no differences in the amount of cAMP synthesised between wild-type and mutant MC4R receptors. Similarly, Fan, Sartin, and Tao (2008) comparing the *in vitro* amount of cellular cAMP produced by wild type and mutant (c.1426G>A) MC4R in pigs, found no evident functional differences between the normal and the mutated receptors.

The expression of melanocortin 4 receptor was assayed in mice by An et al. (2007) who analysed gene expression in different tissues and found that MC4R was highly expressed in the hypothalamus and skeletal muscle but at a very low level in liver and adipose tissue.

The aim of the present research was to study the c.1426G>A polymorphism of the *MC4R* gene in populations of Italian Large White (ILW) and Italian Duroc (IDU) pigs to analyse the relationship between this polymorphism and production traits and carcass quality.

Moreover the expression of MC4R in different pig tissues (liver, lung, brain, muscle, adrenal gland, fat, stomach, kidney) was studied to identify and quantify its expression level.

2. Materials and methods

2.1. Sampling

Allele frequencies of *MC4R* gene, were analysed in a total of 349 unrelated pigs from 11 breeds: 6 cosmopolitan (Italian Large White and Italian Duroc — indicated as "random", Italian Landrace, Pietrain, Belgian Landrace, Hampshire, Pietrain), the Chinese Meishan, and 4 Italian local breeds (Cinta Senese, Casertana, Calabrese, Nero Siciliano). Numbers of pigs per breed are reported in Table 1.

Furthermore, four different pure-breed populations of sib-tested pigs provided by the National Association of Pig Breeders (Associazione Nazionale Allevatori Suini, ANAS; http://www.anas.it), were used: (1) 187 Italian Large White (ILW) pigs selected with extreme estimated breeding value (EBV) for average daily gain; (2) 199 Italian Duroc (IDU) animals selected with extreme EBV for average daily gain; (3) 658 Italian Large White pigs; (4) 404 Italian Duroc. A detailed description of each population is reported in Tables S1a and S1b. Pigs belonging to populations 3 and 4 were randomly chosen. As can be seen in Tables S1a and S1b, the females were approximately double the castrated males in each group and subgroup (pigs with positive and negative EBVs). This particular sex ratio is due to the selection scheme utilised in Italy for a sib-test programme where two females and one castrated male are selected from the same litter of the candidate AI boar for genetic evaluation.

Table 1
Allele frequencies in 11 different pig breeds.

Breeds	No of pigs	Allele frequencies	
		Allele A	Allele G
Italian Large White	62	0.694	0.306
Italian Landrace	56	0.188	0.812
Italian Duroc	61	0.754	0.246
Belgian Landrace	20	0.100	0.900
Hampshire	22	0.841	0.159
Pietrain	31	0.258	0.742
Meishan	9	0.056	0.944
Cinta Senese	15	0.233	0.767
Calabrese	16	0.219	0.781
Nera Siciliana	31	0.532	0.468
Casertana	26	0.269	0.731

As a first step to evaluate associations between polymorphisms in the *MC4R* gene and EBVs a selective genotyping approach investigating if allele frequencies differed between divergent groups of pigs chosen according to their EBV for this trait was applied.

For all the pigs, EBV for average daily gain (ADG; calculated from 30 to 155 kg of live weight with a *quasi ad libitum* feeding level, expressed in grammes), feed conversion ratios (FCR; obtained from feed intake recorded daily and body weight measured bimonthly, expressed in units), backfat thickness (BFT, recorded *post mortem* at the level of the *gluteus medius* muscle, expressed in mm), lean cut weight (LC; the sum of neck and loin weight, expressed in kg) and ham weight (HW, expressed in kg) were calculated and provided by ANAS. Only for the Duroc pigs of groups 2 and 4 was the EBV for visible intermuscular fat (VIF, a categorical trait recorded only in this breed, expressed in units of standard deviation of the probability to transmit intermuscular fat) available. Table S2 reports the observed means ± standard deviation for all the samples belonging to groups 3 and 4. Backfat thickness index is indicated as a difference from the genetic base evaluated as "zero" and this means that the index may present negative values because of selection aimed to moderately reduce BFT. For this reason animals presenting the less negative value of the trait are those with the higher backfat thickness. The visible intramuscular fat (VIF) is an original selection criterion set up and utilised by the Italian pig breeder association (ANAS) in order to reduce, in the Italian Duroc breed, the frequency of the so-called "grassinatura" defect due to an excess of inter and intramuscular fat in thighs. VIF is evaluated subjectively on thigh muscle. The genetic index for this trait indicates the genetic value of the boars for the probability of transmitting this defect (Bosi & Russo, 2004).

For all the analysed pigs, lyophilised blood samples were provided by ANAS and muscle samples were collected at the slaughterhouse in collaboration with ANAS.

2.2. DNA extraction, PCR reaction and analysis of mutation

DNA was extracted from lyophilised blood or muscle using a standard protocol and from lyophilised muscle using the High Pure PCR Template Preparation Kit (Roche Diagnostic GmbH, Mannheim, Germany).

The c.1426G>A mutation was analysed by High Resolution Melting (HRM), a technique based on the melting profile of small amplicons (Liew et al., 2004; Wittwer, Reed, Gundry, Vandersteen, & Pryor, 2003).

To this aim, primers flanking the polymorphism were designed to amplify a 104 bp fragment (forward: 5′-TGTGTGTGCTTCATGTCT-3′, reverse: 5′-TTCCTCAGTTCTTGGCTC-3′). Amplifications were performed with Rotor-Gene™ 6000 (Corbett Research, Mortlake, New South Wales, Australia), in a total volume of 20 μl containing 2 μl of 10X standard buffer, 3 mM $MgCl_2$, 0.3 μM of each primer, 160 μM dNTP, 1 U EuroTaq polymerase (EuroClone S.p.A., Life Science Division, Italy), 1X EvaGreen™ (Biotium Inc., Hayward, CA 94545, USA) and 50–100 ng of template DNA. Cycling conditions were: initial denaturation at 95 °C for 5 min, 40 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s, followed by a final extension step of 72 °C for 2 min. Fluorescence was acquired at the end of each extension step to ensure that all reactions reached the plateau stage. After a holding step at 50 °C for 60 s, HRM analysis was performed by heating the samples from 72 °C to 85 °C, at a rate of 0.2 °C each 20 s, with continuous fluorescence acquisition. HRM data were analysed by Rotor-Gene™ 6000 software. Fluorescence *vs.* temperature plots were normalised by selecting linear regions before and after the melting transition (pre-melting region: 72.0–72.5 °C; post-melting region: 84.0–84.5 °C).

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Genotypes were determined setting known samples as reference and using a reliability threshold of 0.90 for the genotype assignment.

PCR-RFLP protocol was performed to analyse the reference samples and to confirm results obtained by HRM method, using primers designed on a porcine *MC4R* gene fragment as described in Kim, Larsen, and Rothschild (2000) and Kim, Larsen, Short, et al. (2000).

2.3. RNA source, total RNA extraction and cDNA preparation

Total RNA was extracted from frozen tissue (liver, lung, little brain, muscle, adrenal gland, fat, stomach, kidney) samples from three Italian Large White pigs using TRIZOL reagent (Invitrogen Corporation, Carlsbad, California), and then total RNA was quantified and quality assessed both by ND-1000 Spectrophotometer (NanoDrop Technologies, Willmington, DE, USA), with an optimal 260/280 ratio between 1.8 and 2.1, and visualisation on 1% agarose gel. RNA samples were treated with DNase (Invitrogen Corporation) and first-strand cDNA was synthesised using 1 μg total RNA as template (Improm-II™ Reverse Transcription System, Promega Corporation, Milan, Italy) and using 1 μl Oligo-dT primers (Promega Corporation). All reaction conditions were performed as recommended by the manufacturer. To further verify that no genomic DNA was present in each sample, PCR analysis using primers that amplify an intronic sequence of *ACADM* (acyl-CoA dehydrogenase, C-4 to C-12 straight chain) gene was performed.

2.4. Quantification of MC4R expression

Quantitative real-time PCR (qRT-PCR) reactions were performed on a LightCycler 1.0 System (Roche Diagnostics, Mannheim, Germany) using 5 μl of QuantiTech™ SYBR®Green PCR mix (Qiagen, Hilden, Germany), 0.5 μl of the primer used for genotyping at 10 pmol each for *MC4R* (Forward 5′-CCCAGAATCCATACTGTGT-3′; Reverse 5′-TCTTTGAAGGTTTTCCT CAG-3′) and 2 μl of cDNA template. The thermal cycling conditions included an initial hot start at 95 °C for 15 min which activated the conjugated polymerase, followed by 50 cycles of 15 s at 95 °C for DNA denaturation, 30 s at 51 °C for annealing of primers, 18 s at 72 °C for primer extension. Samples were assayed in two replicates. To check the specificity of the amplified products, melting curve analysis was performed and the PCR products were verified by 1% agarose gel-electrophoresis.

For the absolute quantification of *MC4R* expression in skeletal muscle samples, threshold cycles were converted to mRNA molecules/μl using a standard curve (Pfaffl, 2004) generated from serial dilutions (10^8–50 copies/μl) of the PCR amplicon obtained with the primer pair Forward 5′-TACCCTGACCATCTTGATTG-3′; Reverse 5′-ATAGCAACAGATGATCTCTTTG-3′, and containing the annealing region of the primers used in the real-time PCR.

2.5. Statistical analysis for association study

The deviation from Hardy–Weinberg equilibrium of the analysed populations was tested using Fisher's exact test of significance (two tailed) of differences of allele frequency between the group with positive (high) EBVs and the group with negative (low) breeding values was calculated for both ILW and IDU breeds described in groups 1 and 2.

The association analysis using data and genotypes of groups 3 and 4 was performed using the SAS software GLM procedure. The linear model, utilised within each breed (ILW and IDU), was the following:

$$y_{ij} = \mu + MC4R_i + sex_j + \varepsilon_{ij}$$

y_{ij} = EBV for different traits (ADG; LC; BFT; HW; FCR, VIF); μ = EBV means; $MC4R_i$ = genotype fixed effect (i = 1, 2, 3); sex_j = sex fixed effect (j = 1, 2); ε_{ij} = error.

For both breeds the additive genetic effect as half of the difference between the two homozygous groups, and the dominance effect as the difference between the heterozygous group and the average of the two homozygous groups was estimated. The estimates of effects were tested by *t*-test on significant deviation from zero.

Table 2
Allele frequencies at the *MC4R* locus, *P* values of the differences between allele frequencies in the extreme groups of Italian Large White and Italian Duroc pigs for ADG trait (expressed as observed means ± standard deviation).

Breed		No of pigs	Allele frequencies		*P* values	ADG (g)
			A allele	G allele		
ILW	P	91	0.632	0.368	0.005	104.681 ± 8.945
	N	96	0.495	0.505		−50.667 ± 11.246
IDU	P	99	0.919	0.081	<0.001	108.000 ± 39.589
	N	100	0.687	0.313		−27.576 ± 13.067

P = sub-group with extreme positive EBVs; N = sub-group with extreme negative EBVs.

3. Result and discussion

3.1. SNP analysis

In Table 1 allele frequencies of the *MC4R* c.1426G>A SNP obtained analysing 349 pigs of different breeds are reported. *A* allele is the most frequent in the Italian Large White, Italian Duroc and Hampshire pigs, while *G* allele is the most represented in Italian Landrace, Belgian Landrace and Pietrain samples.

The allelic frequencies for the main selected breeds reared in Italy (Italian Large White, Italian Duroc, Pietrain, Landrace) reported in Table 1, are similar to those reported for cosmopolitan breeds reared in other countries (Bruun et al., 2006; Hernández-Sánchez et al., 2003; Houston et al., 2004; Kim, Larsen, & Rothschild, 2000; Kim, Larsen, Short, et al., 2000; Piórkowska et al., 2010; Stachowiak et al., 2006; Van den Maagdenberg et al., 2007) as reported in Table S3.

The allelic frequencies for the local analysed breeds (Cinta Senese, Calabrese and Casertana) showed a high frequency of *G* allele. D'Andrea et al. (2008) observed similar results in a different sample of Casertana pigs (Table S3).

Table 2 reports the allele frequencies at *MC4R* locus in ADG divergent pigs of both populations 1 (ILW) and 2 (IDU). The c.1426G>A polymorphism did not deviate from Hardy–Weinberg equilibrium. In both breeds, significant differences in allele frequencies between pigs with high and low EBV for ADG was found ($P = 0.005$ for ILW and $P < 0.001$ for IDU). The group of animals with the highest EBVs showed a higher frequency of *A* allele. It is noteworthy that for the IDU breed, in the group with positive (high) ADG value *A* allele is almost fixed, as *G* allele is 0.081. On the whole the results of the selective genotyping screening reported in Table 2 showed that *A* allele seems to be associated with a higher growth rate, in agreement with the literature.

Due to this promising results obtained by the selective genotyping approach further samples, randomly chosen from larger groups of ILW and IDU breeds (namely populations 3 and 4, in the material and methods) were analysed to search for association of MC4R with ADG and some important production traits.

Table 3
Allele frequencies in a group of Italian Large White and Italian Duroc pigs utilised for the association analysis.

Breed	No of pigs	Allele frequencies		Genotypic frequencies and counts		
		A allele	G allele	*AA*	*AG*	*GG*
Italian Large White	658	0.664	0.336	0.445 293	0.438 288	0.117 77
Italian Duroc	404	0.770	0.230	0.596 241	0.347 140	0.057 23

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Table 4
Italian Large White population of 658 animals: estimated means ± standard error for each analysed EBV. Pig number for each genotype is reported in brackets.

EBV	Genotype			*P*	Additive effect (*P*)
	AA (293)	AG (288)	GG (77)		
ADG (g)*	29.500 ± 2.806 A	26.468 ± 2.921 a	10.251 ± 5.770 b,B	0.0105	−9.624 ± 3.179 (0.0026)
LC (kg)	1.542 ± 0.181 §	1.629 ± 0.187	2.199 ± 0.351§	0.2376	0.328 ± 0.195 (0.0928)
BFT (mm)	−1.706 ± 0.354 a,A	−2.833 ± 0.366 b,A	−5.118 ± 0.687 B	<0.0001	−1.705 ± 0.381 (<0.0001)
HW (kg)	0.395 ± 0.048 a,§	0.283 ± 0.050 §	0.154 ± 0.094 b	0.0446	−0.120 ± 0.052 (0.0212)
FCR (units)	−0.149 ± 0.018 a,§	−0.107 ± 0.018 §	−0.050 ± 0.034 b	0.0238	0.049 ± 0.019 (0.0098)

Values in the same row with different superscripts are different at the statistical level a, b = $P<0.05$ and A, B = $P<0.01$; § = $P<0.1$; ns = non significant.
* For ADG trait data were available only for 536 pigs.

Allele and genotype frequencies of populations 3 and 4 are reported in Table 3. The allele distribution for each breed did not deviate from Hardy–Weinberg equilibrium.

In population 3 (ILW breed), *MC4R* polymorphism is associated with ADG ($P=0.0105$), FCR ($P=0.0238$), BFT ($P=<0.0001$) and HW ($P=0.0446$). The *AA* genotype showed the highest estimated mean value for ADG trait, the heaviest ham weight, the best feed conversion ratio and the highest backfat thickness with significant differences between animals carrying *GG* and *AA* genotypes (Table 4), in agreement with Van den Maagdenberg et al. (2007). The additive effect of the *A* allele was significant for ADG ($P=0.0026$), FCR ($P=0.0098$), BFT trait ($P<0.0001$) and on HW ($P=0.0212$) (Table 4). No significant dominance effect was present. These findings agree with results for Large White breed homozygous pigs in that *AA* carrying pigs have higher backfat and are faster growing than those carrying the *GG* genotype (Hernández-Sánchez et al., 2003; Houston et al., 2004; Jokubka, Maak, Kerziene, & Swalve, 2006; Kim, Larsen, Short, et al., 2000; Óvilo, Fernández, Rodríguez, Nieto, & Silió, 2006; Van den Maagdenberg et al., 2007).

In the IDU samples the *MC4R* SNP affected ADG ($P=0.0160$), FCR ($P<0.0001$), LC ($P<0.0001$), and HW ($P<0.0001$) EBVs (Table 5). For all the analysed traits, animals carrying the AA genotype had the best estimated mean values compared to GG animals ($P<0.01$). These results are consistent with Kim, Larsen, and Rothschild (2000), Kim, Larsen, Short, et al. (2000) and Hernández-Sánchez et al. (2003) who analysed different synthetic lines (containing also Duroc breed) and found significant effects on ADG, BFT and meat percentage. Additive effects of *A* allele was significant for ADG ($P=0.0053$), FCR ($P<0.0001$), LC ($P=0.0005$), and HW ($P<0.0001$). No significant dominance effect was present.

Comparing ILW and IDU breeds marked differences in the allelic effect on the analysed traits are seen. In the ILW breed, animals carrying the *AA* genotype have higher backfat thickness and lower lean cuts than *GG* pigs. On the contrary, *AA* Italian Duroc animals have heavier LC and thicker BFT compared to EBV estimated mean for animals with the *GG* genotype. Analysis of variance did not highlight an association between the analysed SNP and LC in ILW and with BFT in IDU. Although not significant, the nominal values of the effects of the two alleles for these traits are opposite in the breeds (Tables 4 and 5).

This finding was unexpected because there seems to be a sort of breed specificity. In fact, there seems to be a different metabolic aptitude between breeds to deposit lean and fat tissue.

It is interesting to note that both in the ILW and in the IDU samples, the animals carrying the *GG* genotype were very few (77 out of 658 and 23 out of 404, respectively, Tables 4 and 5) similar to numbers reported elsewhere for other Large White and Duroc pig populations. Schwab et al. (2009) found 24 *GG* pigs out of 333 Duroc animals; Stachowiak et al. (2006) reported the presence of 13 *GG* pigs in192 Large White samples. Bruun et al. (2006), reported over a twelve-year period a significant increase of *A* allele frequency in Duroc pigs reared in Denmark. The low *G* allele frequency in the present study, as in other research (Kim et al., 2006; Van den Maagdenberg et al., 2007), is most likely due to a shifting of the allele frequency from *G* to *A* by selection for daily gain.

The Italian selection schemes applied to IDU and ILW aim to improve the growth rate and feed efficiency, to increase the percentage of lean cuts and reduce backfat thickness (maintaining it at a certain level), in order to provide the proper fat covering of cured products and prevent increased seasoning loss (Bosi & Russo, 2004; Gallo & Buttazzoni, 2008). As a consequence of the relevance put by selection on ADG and considering the significant effect of the *MC4R* gene on this trait, it is possible that the selection programme applied to ILW and IDU populations led to increased *A* allele frequency in both breeds.

Possible explanations for the opposite direction of *MC4R* allelic effects on LC and BFT detected in ILW and IDU samples are as follows.

The simplest interpretation is that c.1426G>A is not the causative mutation but is closely linked to the true functional gene, as reported by Bruun et al. (2006). It is also possible that there is an interaction of the *MC4R* gene (influencing growth and feed intake) with other genes regulating metabolism, energy distribution and storage. This suggested epistatic gene interaction could have effects on nutrient utilisation and could be breed-specific, addressing the growth towards lean or fat deposition depending on the different genetic and metabolic backgrounds of different breeds.

The response to this gene interaction could contribute to the observed divergent trends to lean or fat growth in pigs of different breeds carrying the same genotype at the *MC4R* locus.

ILW and IDU pigs with the *AA* genotype show the highest ADG and also a favourable effect for FCR. In IDU animals ADG is mainly

Table 5
Italian Duroc population of 404 animals: estimated means ± standard error for each analysed EBV. Pig number for each genotype is reported in brackets.

EBV	Genotype			*P*	Additive effect (*P*)
	AA (241)	AG (140)	GG (23)		
ADG (g)*	36.129 ± 2.763 A	30.995 ± 3.257 a	10.940 ± 8.535 b,B	0.0160	−12.594 ± 4.469 (0.0053)
LC (kg)	2.804 ± 0.167 A	1.575 ± 0.214 B	0.897 ± 0.522 B	<0.0001	−0.953 ± 0.273 (0.0005)
BFT (mm)	−2.632 ± 0.260	−1.991 ± 0.333	−1.504 ± 0.814	0.1733	0.564 ± 0.426 (ns)
HW (kg)	1.016 ± 0.061 A	0.465 ± 0.078 B	0.175 ± 0.191 B	<0.0001	−0.421 ± 0.100 (<0.0001)
FCR (units)	−0.209 ± 0.015 A	−0.102 ± 0.020 B,a	0.004 ± 0.048 B,b	<0.0001	0.106 ± 0.025 (<0.0001)
VIF	−0.443 ± 0.087	−0.282 ± 0.112	−0.007 ± 0.273	0.2089	0.218 ± 0.143 (ns)

Values in the same row with different superscripts are different at the statistical level a, b = $P<0.05$ and A, B = $P<0.01$; § = $P<0.1$; ns = non significant.
* For ADG trait data were available only for 234 pigs.

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Table 6
Expression pattern of *MC4R* gene in different porcine tissues.

Tissue	Quantification (copy number/μl)
Liver	ND
Little brain	460
Kidney	ND
Lung	ND
Adrenal gland	ND
Fat	311
Stomach	ND
Skeletal muscle	4

ND = not detected.

addressed towards lean growth. Otherwise in the ILW breed the different genetic metabolic backgrounds may modify the molecular pathway relevant to muscle or fat growth thus influencing the effect of the *A* allele resulting in a higher backfat thickness.

The hypothesis that Asp298 could influence the allocation of nutrients towards lean deposition was reported by Bruun et al. (2006) and Houston et al. (2004) who suggested that the effect on *MC4R* could be line or breed-specific.

Additional studies aimed in better understanding of the molecular interactions involving the *MC4R* gene in processes regulating food intake and lean/fat deposition in ILW and IDU pigs are required.

3.2. Expression profile analysis

In order to verify the presence of transcripts of the *MC4R* gene in pig, RNA from different tissues (liver, little brain, lung, kidney, muscle, adrenal gland, fat, stomach) was analysed by quantitative real time PCR. The results are reported in Table 6 and absolute quantification is expressed as mRNA copy number per μl: it is seen that *MC4R* is expressed little in brain, as expected, and in fat tissue and skeletal muscle, in agreement with An et al. (2007). A very low expression level was found in skeletal muscle tissue (less than 10 molecules/μl) and no expression was detected in the other tissues. A higher expression level was found in porcine fat tissue compared to An et al. (2007). Moreover, a very low expression level was found in skeletal muscle tissue (less than 10 molecules/μl) and no expression was detected in liver. To our knowledge these results show, for the first time in pigs, the presence of the mRNA of this receptor in different tissues in concordance with a previous study on mice: the highest expression was in nervous tissue, a higher expression than reported by An et al. (2007) was detected in fat, and a lower level of expression was detected in skeletal muscle.

4. Conclusions

The c.1426G>A polymorphism showed significant association with ADG, FCR and HW in both Italian Large White and Italian Duroc breeds. However, opposite effects of *MC4R* alleles were found in LC in an Italian Duroc population and in BFT in the ILW pigs suggesting a possible breed specific effect on lean and fat deposition. However it is not excluded that *MC4R* is not the causative mutation but is closely linked to the responsible gene.

Further studies are expected to support the hypothesis that *MC4R* may have a functional role influencing lean and fat growth in pigs.

Supplementary materials related to this article can be found online at doi:10.1016/j.meatsci.2011.11.025.

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References

Adan, R. A. H., Tiesjema, B., Hillebrand, J. J. G., la Fleur, S. E., Kas, M. J. H., & de Krom, M. (2006). The MC4 receptor and control of appetite. *British Journal of Pharmacology*, *149*(7), 815–827.

An, J. J., Rhee, Y., Kim, S. H., Kim, D. M., Han, D. -H., Hwang, J. H., et al. (2007). Peripheral effect of alpha-melanocyte-stimulating hormone on fatty acid oxidation in skeletal muscle. *The Journal of Biological Chemistry*, *282*(5), 2862–2870.

Bell, C. G., Walley, A. J., & Froguel, P. (2005). The genetics of human obesity. *Nature Reviews. Genetics*, *6*(3), 221–234.

Bosi, P., & Russo, V. (2004). The production of the heavy pig for high quality processed products. *Italian Journal of Animal Science*, *3*, 309–321.

Bromberg, Y., Overton, J., Vaisse, C., Leibel, R. L., & Rost, B. (2009). In silico mutagenesis: A case study of the melanocortin 4 receptor. *The FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *23*(9), 3059–3069.

Bruun, C. S., Jørgensen, C. B., Nielsen, V. H., Andersson, L., & Fredholm, M. (2006). Evaluation of the porcine melanocortin 4 receptor (MC4R) gene as a positional candidate for a fatness QTL in a cross between Landrace and Hampshire. *Animal Genetics*, *37*(4), 359–362.

Butler, A. A. (2006). The melanocortin system and energy balance. *Peptides*, *27*(2), 281–290.

Cooper, T. A., Jenkins, S. J., Wojakiewicz, L., Kattesh, H. G., & Kojima, C. J. (2011). Effects of weaning and syndyphalin-33 on expression of melanocortinergic appetite-regulating genes in swine. *Domestic Animal Endocrinology*, *40*(3), 165–172.

D'Andrea, M., Pilla, F., Giuffra, E., Waddington, D., & Archibald, A. L. (2008). Structural analysis and haplotype diversity in swine LEP and MC4R genes. *Journal of Animal Breeding and Genetics = Zeitschrift Für Tierzüchtung Und Züchtungsbiologie*, *125*(2), 130–136.

Fan, Z. -C., Sartin, J. L., & Tao, Y. -X. (2008). Pharmacological analyses of two naturally occurring porcine melanocortin-4 receptor mutations in domestic pigs. *Domestic Animal Endocrinology*, *34*(4), 383–390.

Gallo, M., & Buttazzoni, L. (2008). Duroc Italiana, un elevato potenziale. *Suinicoltura*, *8*, 24–31.

Hernández-Sánchez, J., Visscher, P., Plastow, Graham, & Haley, C. (2003). Candidate gene analysis for quantitative traits using the transmission disequilibrium test: The example of the melanocortin 4-receptor in pigs. *Genetics*, *164*(2), 637–644.

Houston, R. D., Cameron, N. D., & Rance, K. A. (2004). A melanocortin-4 receptor (MC4R) polymorphism is associated with performance traits in divergently selected Large White pig populations. *Animal Genetics*, *35*(5), 386–390.

Hughes, D. A., Hinney, Anke, Brumm, H., Wermter, Anne-Kathrin, Biebermann, H., Hebebrand, Johannes, et al. (2009). Increased constraints on MC4R during primate and human evolution. *Human Genetics*, *124*(6), 633–647.

Jokubka, R., Maak, S., Kerziene, S., & Swalve, H. H. (2006). Association of a melanocortin 4 receptor (MC4R) polymorphism with performance traits in Lithuanian White pigs. *Journal of Animal Breeding and Genetics = Zeitschrift Für Tierzüchtung Und Züchtungsbiologie*, *123*(1), 17–22.

Kim, K. S., Larsen, N. J., & Rothschild, M. F. (2000). Rapid communication: Linkage and physical mapping of the porcine melanocortin-4 receptor (MC4R) gene. *Journal of Animal Science*, *78*(3), 791–792.

Kim, K. S., Larsen, N., Short, T., Plastow, G., & Rothschild, M. F. (2000). A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. *Mammalian Genome: Official Journal of the International Mammalian Genome Society*, *11*(2), 131–135.

Kim, K. S., Lee, J. J., Shin, H. Y., Choi, B. H., Lee, C. K., Kim, J. J., et al. (2006). Association of melanocortin 4 receptor (MC4R) and high mobility group AT-hook 1 (HMGA1) polymorphisms with pig growth and fat deposition traits. *Animal Genetics*, *37*(4), 419–421.

Kim, K. -S., Thomsen, H., Bastiaansen, J., Nguyen, N. T., Dekkers, J. C. M., Plastow, G. S., et al. (2004). Investigation of obesity candidate genes on porcine fat deposition quantitative trait loci regions. *Obesity Research*, *12*(12), 1981–1994.

Liew, M., Pryor, R., Palais, R., Meadows, C., Erali, M., Lyon, E., et al. (2004). Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons. *Clinical Chemistry*, *50*(7), 1156–1164.

Meidtner, K., Wermter, A. -K., Hinney, A., Remschmidt, H., Hebebrand, J., & Fries, R. (2006). Association of the melanocortin 4 receptor with feed intake and daily gain in F2 Mangalitsa × Piétrain pigs. *Animal Genetics*, *37*(3), 245–247.

Óvilo, C., Fernández, A., Rodríguez, M. C., Nieto, M., & Silió, L. (2006). Association of MC4R gene variants with growth, fatness, carcass composition and meat and fat quality traits in heavy pigs. *Meat Science*, *73*(1), 42–47.

Park, H. B., Carlborg, O., Marklund, S., & Andersson, L. (2002). Melanocortin-4 receptor (MC4R) genotypes have no major effect on fatness in a Large White × Wild Boar intercross. *Animal Genetics*, *33*(2), 155–157.

Patten, C. S., Daniels, D., Suzuki, A., Fluharty, S. J., & Yee, D. K. (2007). Structural and signaling requirements of the human melanocortin 4 receptor for MAP kinase activation. *Regulatory Peptides*, *142*(3), 111–122.

Pfaffl, M. W. (2004). Quantification strategy in real time-PCR. In S. A. Bustin (Ed.), *A–Z of quantitative PCR* (pp. 87–112). La Jolla, CA, USA: International University Line (IUL).

Piórkowska, K., Tyra, M., Rogoz, M., Ropka-Molik, K., Oczkowicz, M., & Rózycki, M. (2010). Association of the melanocortin-4 receptor (MC4R) with feed intake, growth, fatness and carcass composition in pigs raised in Poland. *Meat Science*, *85*(2), 297–301.

Schwab, C. R., Mote, B. E., Du, Z. -Q., Amoako, R., Baas, T. J., & Rothschild, M. F. (2009). An evaluation of four candidate genes for use in selection programmes aimed at increased intramuscular fat in Duroc swine. *Journal of Animal Breeding and Genetics = Zeitschrift Für Tierzüchtung Und Züchtungsbiologie*, *126*(3), 228–236.

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Stachowiak, M., Szydlowski, M., Obarzanek-Fojt, M., & Switonski, M. (2006). An effect of a missense mutation in the porcine melanocortin-4 receptor (MC4R) gene on production traits in Polish pig breeds is doubtful. *Animal Genetics*, *37*(1), 55–57.

Tao, Y. -X. (2009). Chapter 6 mutations in melanocortin-4 receptor and human obesity. *Progress in Molecular Biology and Translational Science*, *88*, 173–204.

Tao, Y. -X. (2010). The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. *Endocrine Reviews*, *31*(4), 506–543.

Van den Maagdenberg, K., Stinckens, A., Claeys, E., Seynaeve, M., Clinquart, A., Georges, M., et al. (2007). The Asp298Asn missense mutation in the porcine melanocortin-4 receptor (MC4R) gene can be used to affect growth and carcass traits without an effect on meat quality. *Animal*, *1*(08), 1089–1098.

Wittwer, C. T., Reed, G. H., Gundry, C. N., Vandersteen, J. G., & Pryor, R. J. (2003). High-resolution genotyping by amplicon melting analysis using LCGreen. *Clinical Chemistry*, *49*(6 Pt 1), 853–860.

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