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PAPER

A selective genotyping approach identifies single nucleotide polymorphisms in porcine chromosome 2 genes associated with production and carcass traits in Italian heavy pigs

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Abstract

Several studies have shown that porcine chromosome 2 (SSC2) harbors important quantitative trait loci (QTL) for production traits. In particular, an imprinted QTL for muscle mass production is determined by a mutation in the *IGF2* gene (intron3-g.3072G>A). We recently identified and analysed single nucleotide polymorphisms (SNPs) in genes (cathepsin D, *CTSD* g.70G>A; cathepsin F, CTSF g.22G>C; lactate dehydrogenase A, *LDHA* g.46G>T) localized on SSC2 (including the *IGF2* intron3-g.3072G>A SNP) showing association with production traits in Italian Large White pigs and/or localizing them on QTL regions. Here we analysed these markers applying a selective genotyping approach based on estimated breeding values (EBVs). Three groups of Italian Large White pigs each made by animals with the most positive (n. 50) and most negative (n. 50) EBVs for average daily gain (ADG), backfat thickness (BFT) or weight of lean cuts (LC) and one group of Italian Duroc pigs made by 50 animals with most positive and 50 animals with most negative EBV for visible intermuscular fat (VIF) were genotyped. In Italian Large White pigs, allele frequency differences for the *IGF2* intron3-g.3072G>A SNP between the two extreme tails for all groups were highly significant (considering all analysed animals: P=9.53E-20 for LC; P=3.16E-15 for BFT; P=4.41E-6 for ADG). Significant allele frequency differences were also observed for the *CTSD* g.70G>A (P=0.0002 for ADG; P=0.00068) and *LDHA* g.46G>T (P=2.32E-5 for ADG) polymorphisms. These results provide further support on the effects of these polymorphisms or genes whose application on marker assisted selection programs could be envisaged.

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Introduction

Numerous intercrosses between divergent or commercial pig breeds and lines have been established to identify quantitative trait loci (QTL) associated with major economically important traits. Based on these experiments a large number of QTL have been mapped on all chromosomes (Rothschild *et al*., 2007). In particular on chromosome 2 (SSC2), several studies have reported QTL with large effects on growth rate, meat tenderness, carcass and meat quality traits (e.g.: de Koning *et al*., 2000; Rattink *et al*., 2000; Lee *et al*., 2003; Jungerius *et al*., 2004; Thomsen *et al*., 2004; Vidal *et al*., 2005; Sanchez *et al*., 2006; van Wijk *et al*., 2006; Liu *et al*., 2007, 2008; Tribout *et al*., 2008; Heuven *et al*., 2009b). An imprinted QTL for muscle mass production having effects on several other carcass traits has been localized on the distal end of the p arm of this chromosome (Jeon *et al*., 1999; Nezer *et al*., 1999). Van Laere *et al*. (2003) reported that a polymorphism in the *IGF2* gene (*IGF2* intron3-g.3072G>A) is the responsible mutation for this QTL. This substitution disrupts a repressor nuclear factor binding site in intron 3, causing a three-fold over expression of postnatal skeletal muscle *IGF2* mRNA in pigs inheriting the mutation from their sires, leading to increased muscle mass and, in turn, reduced back fat deposition (Van Laere *et al*., 2003). In addition to this mutation, it appears that the QTL pattern of the *IGF2* region is more complex than previously suggested and it might include non-imprinted QTL (Houston *et al*., 2005). This was demonstrated by a polymorphism in a very close gene to *IGF2*. This marker, identified in the 3'-untranslated region of the cathepsin D (*CTSD*) gene (Russo *et al*., 2008), was shown to be associated with meat production and carcass traits in Italian Large White and Italian Duroc pigs, independently from the effects of the *IGF2*-intron3-g.3072G>A SNP (Fontanesi *et al*., 2010c). About 13 cM from the *CTSD* gene, we assigned the cathepsin F (*CTSF*) gene, a missense mutation of which resulted associated with several production traits in Italian Large White pigs (Russo *et al*., 2004, 2008) but not in Italian Duroc pigs (Fontanesi *et al*., manuscript in preparation). Other reports (Lee *et al*., 2003; Liu *et al*., 2007) have localised QTL for carcass traits in the SSC2 region where we assigned the lactate dehydrogenase A (*LDHA*) gene (Fontanesi *et al*., 2003) and a polymorphism in this gene was shown to be associated with average daily gain in a Berkshire x Yorkshire resource family (Qiu *et al*., 2010).

Selective genotyping for QTL mapping in farm animals has been proposed within families to reduce the cost of genotyping without loosing much power (Darvasi and Soller, 1992). Successful application of this strategy together with DNA pooling has been reported in dairy cattle using a daughter design (Lipkin *et al*., 1998; Mosig *et al*., 2001; Bagnato *et al*., 2008). In these cases, within a large number of daughters of a sire, the extreme divergent daughters for a trait (the two tails of the distribution of its estimated breeding value; EBV), are chosen and genotyped. In pigs, selective genotyping can be applied within populations and lines taking advantage of the large number of animals evaluated in selected nuclei by choosing individuals with extreme high and low EBVs for a given trait (Fontanesi *et al*., 2009; Heuven *et al*., 2009a). This approach could be similar to the case and control exper-

iment designs applied in human genetics studies (Cardon and Bell, 2001) but the differences are that i) in animals it is possible to use EBVs to discriminate two groups and this may maximize genetic differences between them and that ii) extreme tails maximize the distance in terms of trait value compared to case and control studies in which the control group is usually composed of non affected or normal individuals. We recently applied a selective genotyping approach to analyse association between markers in candidate genes and meat production and carcass traits in Italian Large White and Italian Duroc pigs, independently confirming the effects already shown in other association studies (Fontanesi *et al*., 2009, 2010a, 2010b).

Here we selected a few markers in genes mapped on SSC2 (*IGF2*, *CTSD*, *CTSF* and *LDHA*) we already showed to be associated with production traits in Italian pig breeds and/or mapping in QTL regions located on the same chromosome (Fontanesi *et al*., 2003, 2010b; Russo *et al*., 2008). Then we genotyped these markers by using a selective genotyping approach based on EBVs for a few traits in order to evaluate if the results previously obtained could also be confirmed by this experimental design and to evidence other marker/trait associations.

Materials and methods

Animals and traits

This study was conducted on three groups of Italian Large White and one group of Italian Duroc pigs individually performance tested at the Test Station of the National Pig Breeder Association (ANAS). These animals are structured on triplets of siblings from the same litter (two females and one castrated male). Data are used for the genetic evaluation of a boar from the same litter (sib-testing). Each of the three groups of Italian Large White pigs were made by 100 animals chosen among 3591 Italian Large White pigs (evaluated in the period 1996-1999) according to a selective genotyping approach based on EBVs for three different traits (see below for methods of calculation and details about the traits). One group of 100 pigs (69 females and 31 castrated males, 55 of which did not have common parents) had extreme divergent EBVs (50 with the highest and 50 with the lowest EBV) for average daily gain (ADG). Another group of 100 animals (69 females and 31 castrated males, 71 of which did not have common parents) had extreme divergent EBVs (50 with the highest and 50 with the lowest EBV) for back fat thickness (BFT). A third group of 100 pigs (69 females and 31 castrated males, 73 of which had no common parents) had extreme divergent EBVs (50 with the highest and 50 with the lowest EBV) for weight of lean cuts (LC). While 300 extreme EBVs were considered for the three traits (ADG, LC and BFT), only 257 different pigs were genotyped because 43 pigs presented extreme values for more than one trait. The group of Italian Duroc pigs was made by 100 animals (58 females and 42 castrated males, 62 of which did not have common parents) with extreme divergent EBVs (50 with the highest and 50 with the lowest values) for visible intermuscular fat (VIF), chosen among 1225 animals of this breed slaughtered during the same period of the Italian Large White pigs.

Performance and carcass traits

The test period of the animals begun at approximately 30 kg live weight and ended at 155±5 kg live weight. The nutritive level was *quasi ad libitum*. Body weight was measured bimonthly and then daily gain (ADG) was calculated. At the end of the test, the animals of the same testing period were mixed at loading and transported to a commercial slaughterhouse located at 24.5 km far from the Test Station. After the unloading, the pigs were immediately stunned by CO_2 (concentration 87%) using a dip lift system (Butina, Holbæk, Denmark) and bled in a lying position. At the slaughterhouse, within 3 h *post mortem,* BFT at the level of *Musculus gluteus medius*, weight of LC (necks and loins) were measured. VIF is recorded as a categorical trait by trained personnel only in Italian Duroc pigs at 24 h *post mortem* on leg muscles. It resembles intermuscular fat percentage and it is expressed in units of standard deviation of the normal distribution underlying the categorical observed phenotype (ANAS, *http://www.anas.it*).

Analysis of DNA markers

Pig genomic DNA was extracted from blood using a standard protocol. Polymorphisms already identified in the *IGF2* (Van Laere *et al*., 2003), *CTSD*, *CTSF* (Russo *et al*., 2008), and *LDHA* (Fontanesi *et al*., 2003) genes were analysed by PCR-RFLP. Details of the markers and of the genotyping protocols are reported in Table 1. PCR was carried out using a PTC-100 (MJ Research, Watertown, MA, USA) thermal cycler in a final volume of 20 μL that included 10 pmol of each primer, 2.0 mM $MgCl_2$, 2.5 mM each dNTP, 1 U EuroTaq (EuroClone Ltd., Paington, Devon, UK) DNA polymerase. A different PCR protocol was used for the analysis of the *IGF2* polymorphism (Fontanesi *et al*., 2010c). The final volume included 10 pmol of each primer, 2.5 mM $MgSO_4$, 2.5 mM each dNTP, 1 U EuroTaq (EuroClone Ltd., Paington, Devon, UK) DNA polymerase, 0.3X of PCRx Enhancer Solution (PCRx Enhancer System, Invitrogen, Carlsbad, CA, USA) and 1X PCRx AmpBuffer (PCRx Enhancer System, Invitrogen). The PCR profile was the following: an initial step of denaturation for 5 min at 95°C; 35 cycles of 30 s at 95°C, 30 s at the specific annealing temperature for each primer pair (Table 1) and 30 s at 72°C; the final exten-

Table 1. Investigated gene markers, their localization on porcine chromosome 2, PCR analyses and genotyping protocols.

Gene (symbol)	Map position (cM)°	Sequences#	PCR primers (5'-3')"	PCR§	PCR-RFLP analysis^	References
IGF2	5.9	AY242098-AY242112	GACCGAGCCAGGGACGAG *CGCGCCCCACGCGCTCCCACGCTG*	85/62/2.5	*Ade*I: allele g.43A, 23+62 bp; allele g.43G, 85 bp	Van Laere *et al.* (2003); Fontanesi *et al.* (2010)
CTSD	5.9	AM933484 and AM933485	GGCTGTGCACCCTAGGAAC *TCGTCAGGTCCAGGACAAAC*	184/59/2.5	*Msc*I: allele g.70A, 67+117 bp; allele g.70G, 184 bp	Russo *et al.* (2008)
CTSF	19.0	AM933487 and AM933486	AGGGAGGGCTGGAGACGGAGTA TCATTCTGGCTCAGCTCCAC	118/59/2.5	*Rsa*I: allele g.22G, 118 bp; allele g.22C, 97+21 bp	Russo *et al.* (2004, 2008)
LDHA	83.3	AJ557233 and AJ557234	CTATAACGTGACTGCAAACTCTAGG *TTGCAGTTTGGGCTGTATTTT*	150/57/3.5	*Hpy*CH4V: allele g.46G, 150 bp; allele g.46T, 103+47 bp	Fontanesi *et al.* (2003)

IGF2, Insulin-like growth factor 2; *CTSD*, Cathepsin D; *CTSF*, Cathepsin F; *LDHA*, Lactate dehydrogenase A. °Map position of the analysed markers was obtained combining information from Fontanesi *et al*. (2003, 2010a) and Russo *et al*. (2008); #GenBank/EMBL accession numbers of sequences reporting information of the analysed polymorphisms; §PCR conditions: amplified fragment length (bp)/annealing temperature (°C)/[$MgCl_2$] mM; ^restriction enzyme used and PCR-RFLP patterns of the two alleles.

sion step was for 5 min at 72°C. The PCR fragments were digested at 37°C or 65°C overnight using 5 μL of the amplification product and 3 U of the restriction enzymes in a final volume of 25 μL containing 1X enzyme reaction buffer. PCR-RFLP products were electrophoresed on 10% polyacrylamide/bis-acrylamide 29:1 gels and stained with ethidium bromide.

Statistical analysis

Estimated breeding values for average daily gain (ADG, expressed in g), BFT (expressed in mm), LC (expressed in kg), and VIF were predicted by a BLUP-multiple trait animal model including the fixed factors of age at the beginning of test, body weight at slaughter, age at slaughter, day of slaughtering and inbreeding coefficient, besides the random factors of animal and litter. Means and measures of variability of the considered EBVs are reported in Table 2. Fisher's exact test of significance (two tailed) of differences of allele frequency between the two extreme tails of selected pigs was calculated for each trait considering all animals or only animals without common parents. Haplotypes between the *IGF2* and *CTSD* markers were inferred using the PHASE program v. 2.0 (Stephens *et al*., 2001).

Results

Results of the genotyping of the *IGF2*, *CTSD*, *CTSF* and *LDHA* SNPs in the extreme tails of pigs chosen according to their EBVs for ADG, BFT, LC or VIF, are reported in Table 3.

The most significant differences of allele frequencies between the divergent groups have been observed for the *IGF2* intron3-g.3072G>A SNP in the Large White pigs for all three considered traits. Among the Italian Duroc pigs (selected according to their VIF EBV) only one animal was heterozygous for this SNP whereas all other pigs were homozygous for allele intron3-g.3072A. For this reason data obtained at this polymorphic site in this breed were not useful for any comparison. In Italian Large White, allele intron3-g.3072G was the most frequent in the animals with negative ADG EBV (P=4.41E-6, considering all pigs; P=0.031, considering only unrelated pigs at the second generation), in the pigs with positive BFT EBV (P=3.16E-15 and P=1.46E-9, considering all pigs or only unrelated animals at the second generation) and in the animals with negative LC EBV (P=9.53E-20 and P=9.85E-19, for the two comparisons). On the other hand allele intron3-g.3072A was the most frequent in the other tails. These results clearly confirmed the effects of this mutation in affecting lean meat production and carcass traits as already shown in other pig populations and already reported for Italian Large White in a different experiment (Fontanesi *et al*., 2010c). Allele frequencies were in agreement with expected correlations between analysed traits.

Significant differences of allele frequencies were also observed for the *CTSD* g.70G>A polymorphism in the Italian Large White pigs selected according to ADG and LC EBVs. Allele g.70G was more frequent in pigs with negative ADG EBV (P=0.0002 and P=0.0035, either for all animals or for two generation unrelated pigs) and negative LC EBV (P=0.00068 and P=0.00028, in the same subgroups). No significant differences were observed in the two extreme tails of Italian Large White for BFT EBV and in the two divergent tails of Italian Duroc selected according to VIF EBV.

Haplotype analysis of *IGF2* and *CTSD* markers (these genes map very close to each other on SSC2p; Table 1; Fontanesi *et al*., 2010c) in the Italian Large White pigs reported the presence of four haplotypes, three of which (*IGF2* intron3-g.3072G and *CTSD* g.70G, [G:G]; [G:A]; and [A:A]) already identified in this breed (Fontanesi *et al*., 2010c). Their frequency was 10.7%, 31.9%, and 57.0%, respectively (considering all unique Italian Large White pigs from the three groups). The fourth haplotype ([A:G]) was carried by 2 Italian Large White pigs only (frequency of 0.4%) while it was more frequent among the Italian Duroc pigs analysed in this study (7.5%). In this latter breed, only one animal carried haplotype [G:A], whereas the most frequent haplotype was [A:A]. Haplotypes made by these two SNPs were not very informative in evaluating if the two markers play a separate role in affecting the considered traits in Italian Large White pigs because in most cases allele *CTSD* g.70G was associated with allele *IGF2* intron3-g.3072G due to the high linkage disequilibrium between these two markers.

No significant differences in allele frequencies were observed for the *CTSF* g.22G>C polymorphism, even if a higher frequency of allele g.22G in the animals with negative EBV for ADG could be observed. The same allele was associated with lower ADG in another study (Russo *et al*., 2008).

Interestingly, the *LDHA* g.46G>T SNP, located at about 80 cM on the SSC2 linkage map (Table 1; Russo *et al*., 2008) showed significant differences of allele frequencies between the two extreme tails of pigs selected on ADG EBV. Allele g.46T was more frequent in animals with positive EBV both in the complete sample of 100 pigs (P=2.32E-5) and in the reduced sample of two-generation unrelated pigs (P=0.0017). No differences in allele frequencies for this SNP could be observed in the other groups.

Discussion

Selective genotyping approach has been developed to reduce the number of genotyping while maintaining experimental power (Darvasi and Soller, 1992). We applied this experimental design adapting it to the analysis

Table 2. Mean ± standard deviation, minimum and maximum values for estimated breeding values in the four groups of Italian heavy pigs chosen according to a selective genotyping approach.

Trait	Tail°	Pigs, n	EBV, mean ± SD	Min EBV	Max EBV
ADG, g	Negative	50	-60.34±13.12	-110.00	-48.00
	Positive	50	+104.36±13.01	+91.00	+135.00
BFT, mm	Negative	50	-11.40±1.07	-15.80	-10.20
	Positive	50	+10.69±3.19	+8.70	+16.40
LC, kg	Negative	50	-3.74±0.53	-5.98	-3.21
	Positive	50	+5.95±0.49	+5.35	+7.89
VIF	Negative	50	-2.35±0.27	-3.16	-2.00
	Positive	50	+2.17 ± 0.34	+1.75	+2.96

EBV, estimated breeding value; ADG, average daily gain; BFT, backfat thickness; LC, lean cuts; VIF, visible intermuscular fat. The groups for ADG, BFT and LC were of Italian Large White pigs. The group for VIF was of Italian Duroc pigs. °Two extreme tails, each including 50 animals with negative and 50 animals with positive EBVs, for the corresponding traits.

of association with production traits in performance tested Italian heavy pig populations (Italian Large White and Italian Duroc). Effective population size (Ne) of these populations is quite low as they represent nuclei under intensive selection (*data not shown*). Therefore, sample stratification might be considered in order to avoid spurious associations. We managed this issue in an empirical way by including in the analyses all animals from the two tails (100 pigs for each analysed trait) or just a subgroup of two-generation unrelated pigs (Table 3). In both cases we obtained results which confirmed the strong effects already reported for the *IGF2* intron3-g.3072G>A SNP in Italian Large White (Fontanesi *et al*., 2010c) and in other pig populations (Jungerius *et al*., 2004; Estellé *et al*., 2005; Oczkowicz *et al*., 2009). On the other hand, these results indirectly validate the strategy followed in making the two extreme tails and the high reliability of EBVs computed for the Italian Large White population. The ancestral allele (*IGF2* intron3-g.3072G) was associated with higher fat content together with lower muscle mass deposition and lower growth rate. As the effects of this polymorphic site might derive from its imprinted status (Van Laere *et al*., 2003), it could be interesting to evaluate the origin of the two alleles of the analysed pigs. Unfortunately we did not have access to the DNA of the parental animals. The Italian Duroc analysed population was almost completely fixed for allele *IGF2* intron3-g.3072A (only one animal was heterozygous) and this marker was of no use in association analyses. The very low level of heterozygosity of this marker confirms our previous report on Italian Duroc breed (Fontanesi *et al*., 2010c) and what has been indicated for other Duroc populations (Ojeda *et al*., 2008).

The analysis of the *CTSD* g.70G>A polymorphism confirmed that this marker is associated with production traits in Italian Large White pigs, as we previously reported (Fontanesi *et al*., 2010c). The results of selective genotyping for this SNP were significant for ADG and LC but not for BFT. In our previous study conducted on a sample of unselected Italian Large White pigs (random group) the latter trait appeared to be significantly associated with this *CTSD* polymorphic site (Fontanesi *et al*., 2010c). This discrepancy might be due to the high genetic correlations existing among the traits under study: in the previous study on unselected pigs, these correlations could have masked the effects of polymorphism on a single given trait. This confounding effect may be less evident in pigs selected according to extreme EBVs values for the investigated traits (ADG, BFT or LC). As a matter of fact, only 5 and 16 animals with extreme EBVs for ADG also had extreme EBVs values for BFT or LC and only 22 animals with extreme EBVs for BFT had also extreme EBVs for LC. Therefore, results from the selective genotyping approach could have evidenced a direct effect of the *CTSD* marker on biological processes relevant on growth and lean meat deposition. However, in this case two other issues should be considered: i) the effects of linkage disequilibrium with the *IGF2* intron3-g.3072G>A SNP; ii) the low level of heterozygosity of the *CTSD* g.70G>A polymorphism. The high linkage disequilibrium between these SSC2 gene markers in the Italian Large White population prevented the possibility to clearly separate their effects in this study. As a matter of fact almost all animals with the *CTSD* g.70G allele carried in cis the *IGF2* intron3-g.3072G allele (haplotype [G:G]). Separation of the effects of the two polymorphisms was possible in the Italian Duroc population in which allele *IGF2* intron3-g.3072A is almost fixed (Fontanesi *et al*., 2010c). However, in the Italian Duroc pigs chosen according to extreme values for VIF EBV, *CTSD* allele frequencies differences between these two tails were not significant, even if there was a tendency towards a higher frequency of allele g.70G in animals with positive VIF EBV (animals with higher probability to have intermuscular fat; Table 3). In addition, the low level of heterozygosity of the *CTSD* g.70G>A might have had a role in this result. It is worth to point out that the power of the analysis of allele frequency differences in this kind of experiments is proportional to the allele substitution effect, to the level of linkage disequilibrium between the marker and the causative mutation and to the minor allele frequency (Chen *et al*., 2005; Xing and Xing, 2009; Zhang *et al*., 2006).

Results obtained for the *LDHA* SNP indicate that this gene, whose product is involved in the muscle glycolysis pathway, is associated with

Table 3. Allele frequency differences for the analysed SNPs in groups of pigs selected according to extreme estimated breeding values for different traits.

Traits	Groups	Pigs, n	*IGF2*			*CTSD*			*CTSF*			*LDHA*		
			intron3-g.3072G	intron3-g.3072A	P§	g.70G	g.70A	P§	g.22G	g.22C	P§	g.46G	g.46T	P§
ADG	Ne°	50	0.530	0.470	*4.41E-6*	0.180	0.820	*0.0002*	0.360	0.640	*0.128*	0.870	0.130	2.32E-5
	Po°	50	0.210	0.790		0.020	0.980		0.260	0.740		0.600	0.400	
	u Ne#	37	0.541	0.459	*0.031*	0.176	0.824	*0.0035*	0.351	0.649	*0.121*	0.892	0.108	0.0017
	u Po#	18	0.194	0.806		0.000	1.000		0.194	0.806		0.611	0.389	
BFT	Ne°	50	0.180	0.820	*3.16E-15*	0.090	0.910	0.276	0.270	0.730	1	0.890	0.110	0.117
	Po°	50	0.730	0.270		0.150	0.850		0.280	0.720		0.800	0.200	
	u Ne#	35	0.194	0.806	*1.46E-9*	0.083	0.917	0.584	0.292	0.708	0.709	0.889	0.111	0.114
	u Po#	36	0.700	0.300		0.114	0.886		0.257	0.743		0.786	0.214	
LC	Ne°	50	0.810	0.190	*9.53E-20*	0.220	0.780	*0.00068*	0.410	0.590	0.561	0.810	0.190	0.394
	Po°	50	0.180	0.820		0.050	0.950		0.360	0.640		0.750	0.250	
	u Ne#	42	0.810	0.190	*9.85E-19*	0.214	0.786	*0.00028*	0.381	0.619	1	0.798	0.202	0.686
	u Po#	31	0.097	0.903		0.016	0.984		0.371	0.629		0.758	0.242	
VIF	Ne°	50	0.000	1.000	1	0.090	0.910	0.199	0.490	0.510	0.479	0.860	0.140	1
	Po°	50	0.010	0.990		0.160	0.840		0.550	0.450		0.860	0.140	
	u Ne#	29	0.000	1.000	1	0.086	0.914	0.287	0.586	0.414	1	0.845	0.155	0.435
	u Po#	33	0.015	0.985		0.152	0.848		0.576	0.424		0.894	0.106	

ADG, average daily gain; BFT, backfat thickness; LC, lean cuts; VIF, visible intermuscular fat. The groups for ADG, BFT and LC were of Italian Large White pigs. The group for VIF was of Italian Duroc pigs; °animals with negative EBV (Ne) and positive (Po) EBV of the groups of pigs selected according to the extreme and divergent EBV for ADG, BFT, LC or VIF; #unrelated (u) pigs at the second generation with negative EBV (Ne) and positive (Po) EBV of the groups of pigs selected according to the extreme and divergent EBV for ADG, BFT, LC or VIF; §significant results (P<0.05) are in bold. Fisher's exact tests compare the allele frequencies of the groups adjacent to the P-value.

ADG (Table 3). This confirms the results reported by Qiu *et al*. (2010) who, analysing a different polymorphism in this gene, obtained the same result in an F2 experimental population, making *LDHA* a candidate gene for growth performances.

Conclusions

Selective genotyping approach used in this study was successful in identifying gene markers associated with ADG, BFT, and LC. The results for 3 out of 4 polymorphisms (identified in *IGF2*, *CTSD* and *LDHA* genes) confirm previous studies on their effects on several meat production and carcass traits. In particular, in Italian Large White pigs, allele frequency differences between the two alleles of the *IGF2* intron3-g.3072G>A SNP in the two extreme tails were highly significant for all analysed traits. The balanced frequency of the two alleles in the analysed population and the high allele substitution effects of this marker probably contributed to these results. Several QTL have been located on SSC2. Genotyping a larger number of SNPs by using a selective genotyping approach would help in defining main QTLs segregating in the considered populations. Applications of marker assisted selection programs based on results obtained or confirmed with this approach could be envisaged.

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