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A genomewide association study for average daily gain in Italian Large White pigs[1]

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ABSTRACT: Average daily gain is an important target trait in pig breeding programs. In this study we performed a genomewide association study for ADG in Italian Large White pigs using a selective genotyping approach. Two extreme and divergent groups of Italian Large White pigs (number 190 + 190) were selected among a population of about 10,000 performance tested gilts (EBV for ADG in the 2 groups were –30 ± 14 g and 81 ± 12 g, respectively) and genotyped with the Illumina PorcineSNP60 BeadChip. Association analysis was performed treating the pigs of the 2 extreme groups as cases and controls after correction for family-based stratification. A total of 127 SNP resulted significantly associated with ADG (P nominal value [P_{raw}] < 2.0×10^{-7}, $P < 0.01$ Bonferroni corrected [$P_{Bonferroni}$] < 0.01, false discovery rate < 7.76×10^{-5}). Another 102 SNP were suggestively associated with the target trait (P_{raw} between 2.0×10^{-7} and 2.02×10^{-6}, $P_{Bonferroni} < 0.10$, false discovery rate < 4.19×10^{-4}). These SNP were located on all autosomes and on porcine chromosome (SSC) X. The largest number of SNP within this list was on SSC5 ($n = 42$), SSC7 (34), SSC6 (30), SSC4 (23), and SSC16 (16). These chromosomes were richer in significant or suggestively significant markers than expected ($P < 0.001$). A quite high number of these SNP ($n = 23$) were associated with backfat thickness in a previous genomewide association study performed in the same pig population, confirming the negative correlation between the 2 traits. Two or more SNP targeted the same gene: *IGSF3* and *HS2ST1* (SSC4), *OTOGL* (SSC5), *FTO* region (SSC6), and *MYLK4* and *MCUR1* (SSC7). Other regions that were associated with ADG in previous candidate gene studies (e.g., *MC4R* on SSC1, *IGF2* and *LDHA* on SSC2, *MUC4* on SSC13) 1) included markers with $P_{raw} < 0.01$ that, however, did not pass the stringent threshold of significance adopted in this study or 2) could not be tested because not assigned to the Sscrofa10.2 genome version. Functional annotation of the significant regions using Gene Ontology suggested that many and complex processes at different levels are involved in affecting ADG, indicating the complexity of the genetic factors controlling this ultimate phenotype. The obtained results may contribute to understand the genetic mechanisms determining ADG that could open new perspectives to improve selection efficiency in this breed.

Key words: average daily gain, genomewide association study, Italian Large White pigs, selective genotyping, single nucleotide polymorphism

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INTRODUCTION

Growth rate, measured at different growth stages, is an important objective in pig breeding programs as it is directly related to economic advantages. Therefore, measures of this phenotype, such as ADG, are usually included as target traits in selection programs in purebred and commercial pig lines.

Quantitative trait loci for growth performances and related traits have been reported on almost all porcine chromosomes (Hu et al., 2013), suggesting that growth efficiency is a complex trait determined by a large number of loci. In addition, candidate genes have been associated with ADG in different pig populations, including Italian heavy pig breeds (e.g., Fontanesi et al., 2010b, 2011, 2012a,b).

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Several of our previous association studies between DNA markers and production traits in these breeds were based on a selective genotyping strategy in which only the most extreme animals for the target trait, selected within a large performance tested population, were genotyped. This method provided a cost-effective and powerful experimental design (Darvasi and Soller, 1992) to identify gene associated with economically important traits in genetically evaluated pigs of nucleus herds (Fontanesi et al., 2009, 2012a,b,c,d).

The recent development of a high throughput commercial genotyping platform in pigs (Porcine-SNP60 Genotyping BeadChip, Illumina inc. San Diego, CA; Ramos et al., 2009) that can analyze more than 60,000 SNP throughout the pig genome now enables us to perform genomewide association studies (**GWAS**), improving efficiency in detecting genome regions affecting production traits. We already performed a GWAS in Italian Large White pigs for backfat thickness (**BFT**) and identified novel chromosome regions affecting fat deposition (Fontanesi et al., 2012d).

In this work we performed a GWAS for ADG in Italian Large White using a selective genotyping approach and identified SNP associated with this trait adding information about the genetic complexity affecting growth performances in pigs.

MATERIALS AND METHODS

All animals used in this study were kept according to Italian and European legislation for pig production and all procedures described were in compliance with national and European Union regulations for animal care and slaughtering.

Animals

The association study was conducted following a selective genotyping approach, as already described (Fontanesi et al., 2012c,d). Briefly, 2 extreme and divergent groups of Italian Large White gilts, identified within a population of about 10,000 pigs performance tested in the period 1996 through 2009, were used in this study. These animals were included in the national selection program of the Italian Large White breed. This program is based on triplets of siblings from the same litter, 2 females and 1 castrated male that are individually performance tested at the Central Test Station of the National Pig Breeder Association for the genetic evaluation of a boar from the same litter (sib testing). This population is virtually free from the *RYR1* c.1843T allele (Fontanesi et al., 2008, 2012c). Performance evaluation starts when the pigs are 30 to 45 d of age and it ends when the animals reach 155 ± 5 kg live weight. The nutritive level is quasi ad libitum, meaning that about 60% of the pigs are able to ingest the entire supplied ration (Fontanesi et al., 2010b). During the performance test period, body weight of the pigs is measured every 15 d after fasting, and then daily gain is calculated using body weight regress on the repeated test day. At the end of test, animals are transported to a commercial abattoir where they are slaughtered following standard procedures. The extreme and divergent gilts were chosen according to their relatedness and their EBV for ADG: 1) all gilts, among a population of about 10,000 performance tested gilts, were ranked according to their EBV for ADG, 2) among the animals related at 2-generation levels, only the most extreme gilt (with most positive or most negative EBV for ADG) was selected, 3) this procedure selected 190 gilts with the most negative and 190 gilts with the most positive EBV not related at 2-generation levels, and 4) average EBV for ADG in the negative and positive selected groups of pigs were –30 ± 14 g (mean ± SD; minimum: –76 g; maximum: –9 g) and 81 ± 12 g (minimum: 69 g; maximum: 129 g), respectively.

Genotyping

Blood was collected from all performance tested animals and then dried. Dried blood of chosen gilts was used to extract genomic DNA applying standard protocols. After quality control, 375 animals were used for genotyping using the Illumina PorcineSNP60 BeadChip (Ramos et al., 2009) according to the manufacturer's protocol.

Data Analyses

Estimated breeding values for ADG were calculated in the whole performance tested population in 2010 using a BLUP-Multiple Trait-Animal Model. The model included the fixed effect of sex (considering the triplets of pigs from the same litter), batch on trial, inbreeding coefficient of the animal, interaction of sex × age at slaughtering, and date of slaughtering and random effect of litter and animal. The following criteria were used to filter animals and SNP before association analysis: call rate > 0.9 (both at the animal and SNP level) and minor allele frequency (**MAF**) > 0.05. Association analysis was performed treating the pigs of the 2 extreme groups as cases and controls, using the 2 groups of animals with divergent ADG EBV. To detect and correct for possible genetic substructure in the experimental design adopted (Fontanesi et al., 2012d), association tests were performed according to the method for single marker association proposed by Price et al. (2006). For the n animals involved in the study, the $n \times n$ kinship matrix **K** was estimated starting from available pedigree information. Classical multidimensional scaling was applied on 0.5 –

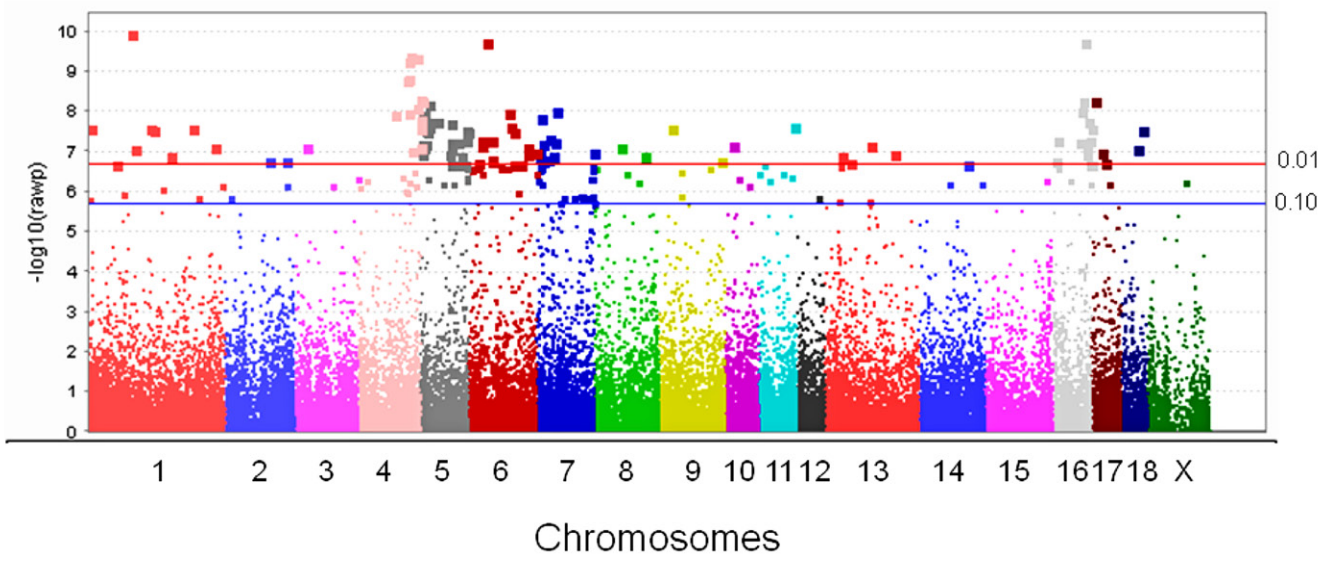

Figure 1. Manhattan plot of SNP in the genomewide association study with ADG in Italian Large White pigs. Red line: $P < 0.01$ Bonferroni corrected ($P_{\text{Bonferroni}}$) = 0.01; blue line: $P_{\text{Bonferroni}} = 0.10$.

K (which acts as a pairwise distance matrix) to identify a number $D << n$ of first axes describing as much genetic difference among animals as possible. Let $c_1,\ldots,c_D$ denote these D axes of genetic variation. Adjustment for possible family-based stratification was performed by regressing the genotype at the ith SNP and the phenotype onto the D continuous axes and taking the corresponding regression residuals as corrected genotypes and phenotypes, respectively. Namely, let g_{ij} and p_j be the genotype at SNP i ($g_{ij} = 0$, 1 or 2) and the phenotype of animal j, respectively. The adjustment was performed on genotypes and phenotype according to the following formulas:

$$g_{ij}^{*} = g_{ij} - \hat{\beta}_{1i}c_{1j} - \ldots. - \hat{\beta}_{Di}c_{Dj}, \text{ and}$$

$$p_j^{*} = p_j - \hat{\gamma}_1 c_{1j} - \ldots. - \hat{\gamma}_D c_{Dj},$$

in which c_{dj} is the score of animal j along the dth axis of genetic variation and $\hat{\beta}_{di}$ and $\hat{\gamma}_d$ are the corresponding estimated partial regression coefficients. These coefficients were obtained using multiple regression models for predicting the ith genotype and the phenotype, respectively, on the basis of the D axes.

The association test statistic is computed as $(n-D-1)r_i^2$, in which

$$r_i^2 = \left(\sum_{j=1}^{n} p_j^{*} g_{ij}^{*}\right)^2 / \sum_{j=1}^{n}\left(p_j^{*}\right)^2 \sum_{j=1}^{n}\left(g_{ij}^{*}\right)^2$$

is the squared correlation coefficient between the ith adjusted genotype and the adjusted phenotype. As remarked by Price et al. (2006), this statistic is a generalization of the Armitage trend statistic usually adopted for categorical genotypes and phenotypes.

Supplementary Fig. 1 shows enrichment of low P nominal values beyond what would be expected under a uniform distribution.

Single nucleotide polymorphisms with P nominal value ($\boldsymbol{P}_{\text{raw}}$) < 2.0×10^{-7} ($P < 0.01$ Bonferroni corrected [$\boldsymbol{P}_{\text{Bonferroni}}$]) were considered significantly associated with ADG. The corresponding false discovery rate (**FDR**) was equal to 7.76×10^{-5} (Benjamini and Hochberg, 1995). Single nucleotide polymorphisms with P_{raw} values between 2.0×10^{-7} and 2.02×10^{-6} ($P_{\text{Bonferroni}} = 0.10$, FDR < 4.19×10^{-4}) were considered suggestively associated with the target trait. For each chromosome, the expected proportion of SNP with $P_{\text{Bonferroni}} < 0.10$ was computed under the assumption of uniform distribution from the informative SNP over the chromosome. This proportion was compared to the proportion of significant or suggestively significant markers actually observed on the same chromosome. Proportion of phenotype variance explained by each significant SNP was not calculated as the selective genotyping design would produce a biased estimation.

All analyses were performed in R (R Development Core Team, 2013). Package kinship2 (Therneau et al., 2012) was used to compute the pedigree-based kinship matrix; package GenABEL (Aulchenko et al., 2007) was used to perform association tests.

Bioinformatics Analyses

Mapping of the PorcineSNP60 BeadChip SNP was obtained on the Sscrofa10.2 genome assembly as previously described (Fontanesi et al., 2012c,d). Significant unassigned SNP in the Sscrofa10.2 were mapped on the Sscrofa9.2 genome version. Identification of the closest genes

to SNP with $P_{Bonferroni} < 0.10$ was obtained using Ensembl annotation of Sscrofa10.2 genome version (July 2013) and Biomart (www.ensembl.org/biomart/martview/). For subsequent analyses, a window of 100 kb in 5′ and 100 kb in 3′ of the SNP in this list was used to retrieve additional genes close to the significant or suggestively significant markers. This window of 0.2 Mb can be considered a conservative approach that can be easily extended using coordinate systems reported in this study. Starting from the corresponding protein sequences retrieved from these databases, the corresponding gene symbols were extracted from National Center for Biotechnology Information gene section (www.ncbi.nlm.nih.gov/gene/) and/or Uniprot (www.uniprot.org/) databases(July 2013). Gene annotation was verified by basic local alignment search tool (BLAST) analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Gene Ontology (**GO**) analysis was performed using DAVID Bioinformatics Resources 6.7 (http://david.abcc.ncifcrf.gov/; Huang et al., 2009) using information about the closest gene.

RESULTS AND DISCUSSION

Genotyping Data

Of the 375 genotyped animals, 5 were excluded from further analysis because their call rate was <0.90. After filtering the 62,163 SNP of the Illumina PorcineSNP60 BeadChip (750 SNP had call rate <0.90 and 12,000 SNP had MAF <0.05), a total of 49,413 SNP were used for subsequent analyses. These SNP were remapped on the Sscrofa10.2 genome version: 42,885 were assigned to assembled porcine chromosomes in only 1 position, 6,528 were assigned to unassembled scaffolds, and 2,938 were not assigned (or were assigned to more than 1 position and were not considered as uniquely mapped).

Genome Scan Results

Figure 1 reports a Manhattan plot showing significant and suggestively significant SNP. A total of 127 SNP resulted significantly associated with ADG (Table 1; Supplementary Table 1).

Another 102 SNP resulted suggestively associated with the target trait (Supplementary Table 1). Among the 229 SNP associated or suggestively associated with ADG, only 8 were not assigned to any chromosome and 4 were placed in unassigned scaffolds of the Sscrofa10.2 genome version. Mapped SNP were located on all autosomes and on porcine chromosome (SSC) X. The largest number of the SNP within this list was on SSC5 (number of SNP with $P_{Bonferroni} < 0.10 = 42$ and number of SNP with $P_{Bonferroni} < 0.01 = 31$), SSC7 (number of SNP considering the 2 thresholds were 34 and 13), SSC6 (30 and 20 SNP, respectively), SSC4 (23 and 14 SNP, respectively) and SSC16 (16 and 13 SNP, respectively; Supplementary Table 1). These chromosomes were richer in significant or suggestively significant markers than expected ($P < 0.001$; SSC4: expected proportion = 0.067, observed = 0.111; SSC5: expected = 0.045, observed = 0.203; SSC6: expected = 0.060, observed = 0.145; SSC7: expected = 0.063, observed = 0.164; and SSC16: expected = 0.035, observed = 0.077). A large number of QTL for ADG and growth performances have been already reported on these chromosomes. For example, on July 2013 (release 20) the PigQTLdb (www.animalgenome.org/cgi-bin/QTLdb/SS/index) reports 45, 9, 25, 36, and 3 QTL for ADG on SSC4, SSC5, SSC6, SSC7, and SSC16, respectively. *Sus scrofa* chromosome 4, SSC6, and SSC7 were among the richest chromosomes of significant SNP in our previous GWAS for BFT in Italian Large White pigs (Fontanesi et al., 2012d), suggesting that several regions identified in our previous GWAS and the current study might contain QTL with pleiotropic effects on both traits. Twenty-three of the 229 SNP identified for ADG in the current study were also previously reported to be significant or suggestively significant for BFT in the same pig population (Fontanesi et al., 2012d; Table 1; Supplementary Table 1). Moreover, several other SNP associated with ADG in this study are close to the markers associated with BFT studied before, and the direction of the effects was opposite (Fontanesi et al., 2012d). This could be expected as EBV for ADG and BFT in Italian Large White pigs are negatively correlated ($r^2 = –0.44$; Fontanesi et al., 2013).

The most significant SNP (ALGA0030787, $P_{raw} = 3.19 \times 10^{-11}$) was not mapped in Sscrofa10.2 even if it was assigned to SSC5 (position 11032453) in Sscrofa9.2. The second and third most significant SNP (ALGA0004718, $P_{raw} = 1.04 \times 10^{-10}$, and ALGA0004837, $P_{raw} = 1.17 \times 10^{-10}$) were localized on SSC1. Other highly significant SNP ($P_{raw} < 1.00 \times 10^{-9}$) were identified on SSC4 (M1GA0006302, M1GA0006343, and M1GA0006613), SSC6 (ALGA0035254), and SSC16 (M1GA0021128; Table 1).

Several chromosome regions included 3 or more SNP ($P_{Bonferroni} < 0.10$) separately to each other by less than 1.5 Mb (Supplementary Table 1). In particular, 2 regions with these features were identified on SSC4 (111.51–114.17 and 141.17–143.22 Mb), 5 on SSC5 (2.61–3.09, 8.03–9.29, 65.43–67.62, 72.59–74.37, and 105.35–109.06 Mb), 4 on SSC6 (26.20–30.90, 50.50–50.85, 91.14–92.90, and 100.66–101.77 Mb), 2 on SSC7 (7.54–11.61 and 128.67–130.84 Mb), and 1 on SSC16 (80.84–82.68 Mb), with significant SNP in the middle or close to these regions (including blocks with 2 closely spaced SNP) that might reflect the presence of different haploblocks (L. Fontanesi, personal communication).

Table 1. Significant SNP associated with ADG in Italian Large White pigs with information about the closest gene as reported in Sscrofa10.2

SNP [1]	SSC	Position	P_{raw} [2]	$P_{Bonferroni}$ [3]	Gene symbol [4]	Distance, bp [4]	SNP [1]	SSC	Position [5]	P_{raw} [2]	$P_{Bonferroni}$ [3]	Gene Symbol [4]	Distance, bp [4]
ALGA0000014*	1	56,5627	2.30×10^{-8}	1.14×10^{-3}	*CH242-271M12.1*	72,271	M1GA0008525	6	47,265,406	4.76×10^{-8}	2.35×10^{-3}	*BCL3*	97,353
ALGA0003521	1	60,455,614	1.82×10^{-7}	9.01×10^{-3}	*DDX43*	1,041,253	M1GA0008519	6	47,321,053	4.76×10^{-8}	2.35×10^{-3}	*PRR2*	Intragenic
ALGA0004718*	1	94,737,894	1.04×10^{-10}	5.15×10^{-6}	Novel pseudogene	36,318	M1GA0008536	6	50,495,796	1.50×10^{-7}	7.43×10^{-3}	*RRAS*	82,042
ALGA0004837	1	99,300,150	1.17×10^{-10}	5.78×10^{-6}	*HTR1B*	165,442	M1GA0008537	6	50,638,891	1.45×10^{-7}	7.15×10^{-3}	*AP2A1*	85,602
ALGA0004958	1	102,500,451	7.79×10^{-8}	3.85×10^{-3}	*MB21D1*	1,035,398	M1GA0008539	6	5,084,7065	4.76×10^{-8}	2.35×10^{-3}	*VRK3*	96,686
ASGA0096650	1	137,394,981	2.50×10^{-8}	1.24×10^{-3}	*FBN1*	91,415	ALGA0036150	6	91,513,570	1.01×10^{-8}	5.00×10^{-4}	-	22,354
ALGA0005986	1	145,646,235	2.74×10^{-8}	1.35×10^{-3}	*DLL4*	97,416	M1GA0008850	6	92,900,402	2.11×10^{-8}	1.04×10^{-3}	-	47,209
ALGA0006831	1	184,400,666	1.11×10^{-7}	5.50×10^{-3}	*CORO2B*	Intragenic	M1GA0008859*	6	100,662,783	3.01×10^{-8}	1.49×10^{-3}	*CABLES1*	Intragenic
ALGA0007846*	1	238,616,307	2.30×10^{-8}	1.14×10^{-3}	-	1,025,259	M1GA0008862*	6	101,205,770	1.87×10^{-7}	9.23×10^{-3}	*LAMA3*	Intragenic
ALGA0009614	1	287,443,993	6.74×10^{-8}	3.33×10^{-3}	*TRIM32*	1,006,260	M1GA0008864*	6	101,772,766	1.87×10^{-7}	9.23×10^{-3}	*OSBPL1A*	Intragenic
ALGA0014534	2	98,997,793	1.61×10^{-7}	7.94×10^{-3}	*MEF2C*	112,521	M1GA0008893*	6	119,164,257	1.87×10^{-7}	9.23×10^{-3}	*PDZK1IP1*	84,766
ALGA0016010	2	137,184,334	1.59×10^{-7}	7.85×10^{-3}	*SLC27A6*	9,625	DRGA0006924	6	132,658,334	1.10×10^{-7}	5.45×10^{-3}	*LRRC7*	45,971
ALGA0018040	3	23,224,453	6.75×10^{-8}	3.33×10^{-3}	*LOC100522792*	49,643	DRGA0006951	6	134,411,824	7.30×10^{-8}	3.61×10^{-3}	*SLC35D1*	29,042
ALGA0025924	4	80,363,802	1.08×10^{-8}	5.32×10^{-4}	*RAB2A*	1,080,804	M1GA0009091	6	155,139,641	9.80×10^{-8}	4.84×10^{-3}	*LOC100737128*	61,610
M1GA0006238	4	111,511,647	1.47×10^{-9}	7.26×10^{-5}	*HMGCS2*	8,1005	M1GA0009342	7	1,611,710	1.22×10^{-7}	6.01×10^{-3}	*LOC100157526*	62,661
M1GA0006250	4	112,746,036	9.82×10^{-9}	4.85×10^{-4}	*LOC100155374*	Intragenic	M1GA0009339	7	1,780,404	9.80×10^{-8}	4.84×10^{-3}	*LOC100157526*	90,238
M1GA0006299*	4	114,151,582	1.30×10^{-9}	6.44×10^{-5}	*IGSF3*	Intragenic	M1GA0009374	7	3,571,588	9.80×10^{-8}	4.84×10^{-3}	*FARS2*	23,283
M1GA0006302*	4	114,170,369	4.84×10^{-10}	2.39×10^{-5}	*IGSF3*	4,195	M1GA0009455	7	5,113,060	9.80×10^{-8}	4.84×10^{-3}	*LOC100156744*	91,839
M1GA0006343*	4	116,750,464	3.57×10^{-10}	1.76×10^{-5}	*OLFML3*	43,229	M1GA0009500	7	7,664,403	1.27×10^{-8}	6.28×10^{-4}	*LOC100738362*	32,601
M1GA0006478	4	122,816,731	8.35×10^{-8}	4.13×10^{-3}	*LOC100737105*	Intragenic	M1GA0009555	7	1,0031,634	1.49×10^{-7}	7.34×10^{-3}	*LOC100739137*	52,139
M1GA0006616	4	134,698,568	7.21×10^{-9}	3.56×10^{-4}	*LOC100152734*	32,777	M1GA0009568	7	1,0543,393	5.71×10^{-8}	2.82×10^{-3}	*CCDC90A*	5,345
M1GA0006613	4	134,796,609	4.32×10^{-10}	2.13×10^{-5}	*LOC100155583*	26,428	M1GA0009677	7	21,031,127	1.41×10^{-7}	6.97×10^{-3}	*CMAHP*	1,162
M1GA0006828	4	141,168,185	2.40×10^{-8}	1.18×10^{-3}	*CLCA2*	1,020,511	M1GA0009735	7	25,272,090	4.20×10^{-8}	2.07×10^{-3}	*MOG*	79,658
M1GA0006854	4	141,458,072	7.05×10^{-8}	3.48×10^{-3}	*HS2ST1*	92,125	M1GA0009865	7	33,358,569	1.16×10^{-7}	5.75×10^{-3}	*BEND6*	85,410
M1GA0006869	4	141,552,372	4.36×10^{-9}	2.16×10^{-4}	*HS2ST1*	Intragenic	M1GA0010028	7	37,366,668	5.42×10^{-8}	2.68×10^{-3}	*CDKN1A*	99,505
M1GA0006938	4	142,907,745	1.43×10^{-8}	7.06×10^{-4}	*ZNHIT6*	32,699	ALGA0040777	7	41,624,144	9.12×10^{-9}	4.51×10^{-4}	*LOC100518497*	6,957
M1GA0006965	4	143,224,099	4.66×10^{-9}	2.30×10^{-4}	*DDAH1*	17,835	M1GA0011382	7	130,525,474	9.49×10^{-8}	4.69×10^{-3}	-	1,013,453
M1GA0007072	5	844,337	1.08×10^{-8}	5.32×10^{-4}	*ATXN10*	Intragenic	M1GA0011548	8	723,623	4.54×10^{-8}	2.24×10^{-3}	*WHSC2*	96,776
M1GA0007246*	5	2,608,692	6.34×10^{-8}	3.13×10^{-3}	*SULT4A1*	60,112	ALGA0047898	8	56,440,088	7.30×10^{-8}	3.61×10^{-3}	*REST*	1,000,471
M1GA0007255	5	2,683,343	9.98×10^{-8}	4.93×10^{-3}	*5S_rRNA*	10,703	ALGA0048976	8	112,525,755	1.16×10^{-7}	5.75×10^{-3}	*SYNPO2*	Intragenic
M1GA0007258*	5	2,748,616	5.73×10^{-8}	2.83×10^{-3}	*EFCAB6*	Intragenic	ASGA0042165	9	27,635,662	2.32×10^{-8}	1.15×10^{-3}	-	1,118,578
M1GA0007286	5	3,095,194	1.06×10^{-7}	5.26×10^{-3}	*SCUBE1*	Intragenic	ALGA0055314	9	139,257,822	1.58×10^{-7}	7.83×10^{-3}	*U6*	26,534
M1GA0007352	5	4,855,248	1.20×10^{-8}	5.95×10^{-4}	*XPNPEP3*	91,269	ALGA0057214	10	15,467,849	6.62×10^{-8}	3.27×10^{-3}	*CNIH3*	81,000
ALGA0030091	5	5,363,246	6.75×10^{-8}	3.33×10^{-3}	*ADSL*	85,183	H3GA0032476	11	77,940,001	2.07×10^{-8}	1.02×10^{-3}	*FGF14*	99,934
M1GA0007436	5	6,942,486	1.12×10^{-8}	5.54×10^{-4}	*CSNK1E*	39,565	H3GA0036210	13	36,964,009	1.19×10^{-7}	5.90×10^{-3}	*RBM15B*	70,931
M1GA0007494*	5	8,031,569	2.23×10^{-8}	1.10×10^{-3}	*CARD10*	65,189	DRGA0012382	13	56,900,313	1.65×10^{-7}	8.16×10^{-3}	-	57,664
M1GA0007506	5	8,303,062	2.74×10^{-8}	1.35×10^{-3}	*C1QTNF6*	99,276	DRGA0012768	13	103,171,133	6.39×10^{-8}	3.16×10^{-3}	*MME*	42,849
M1GA0007538	5	9,292,551	5.57×10^{-8}	2.75×10^{-3}	*LOC100517940*	16,264	ALGA0072425	13	159,407,802	1.05×10^{-7}	5.19×10^{-3}	*U6*	20,127

continued

Table 1 (cont.)

SNP [1]	SSC	Position	P_{raw} [2]	$P_{Bonferroni}$ [3]	Gene symbol [4]	Distance, bp [4]	SNP [1]	SSC	Position [5]	P_{raw} [2]	$P_{Bonferroni}$ [3]	Gene Symbol [4]	Distance, bp [4]
M1GA0007600	5	11,249,463	2.08×10^{-8}	1.03×10^{-3}	*SYN3*	1,011,272	ALGA0080306	14	10,7939,105	1.85×10^{-7}	9.16×10^{-3}	*A1CF*	90,844
M1GA0007630	5	14,225,240	2.78×10^{-8}	1.37×10^{-3}	*LOC100620963*	21,192	ALGA0088670	16	4,016,044	1.59×10^{-7}	7.85×10^{-3}	*TRIO*	16,110
M1GA0007662	5	16,120,363	6.18×10^{-9}	3.06×10^{-4}	*AQP6*	49,433	ALGA0088909	16	7,471,376	4.76×10^{-8}	2.35×10^{-3}	*FAM134B*	1,146,830
M1GA0007707	5	18,676,679	2.18×10^{-8}	1.08×10^{-3}	*KRT4*	84,202	ALGA0090834	16	58,445,833	5.02×10^{-8}	2.48×10^{-3}	*FAM196B*	41,336
M1GA0007772	5	30,095,581	1.66×10^{-8}	8.20×10^{-4}	*MON2*	85,519	M1GA0021097	16	59,838,300	8.72×10^{-9}	4.31×10^{-4}	*SLIT3*	47,859
M1GA0007784	5	35,802,016	1.66×10^{-8}	8.20×10^{-4}	*LOC100152555*	65,262	ALGA0091161	16	66,890,995	4.89×10^{-9}	2.42×10^{-4}	*LOC100516706*	10,282
M1GA0007824	5	65,432,242	1.09×10^{-7}	5.40×10^{-3}	*PHC1*	54,480	M1GA0021128	16	69,042,784	1.61×10^{-10}	7.94×10^{-6}	*LOC100519063*	38,294
M1GA0007840	5	66,740,501	7.96×10^{-8}	3.93×10^{-3}	*VAMP1*	92,758	M1GA0021136	16	73,257,168	8.97×10^{-8}	4.43×10^{-3}	*C5ORF4*	1,021,984
M1GA0007853	5	67,619,158	1.84×10^{-8}	9.11×10^{-4}	*KCNA5*	34,256	ALGA0091438	16	73,552,942	1.09×10^{-7}	5.38×10^{-3}	*HAND1*	1,036,732
M1GA0007928	5	69,437,477	1.97×10^{-7}	9.75×10^{-3}	*TSPAN9*	43,044	M1GA0021168	16	77,794,570	1.66×10^{-8}	8.20×10^{-4}	*FAT2*	34,727
M1GA0007944	5	69,759,629	5.35×10^{-8}	2.64×10^{-3}	*SLC6A12*	73,043	M1GA0021255	16	80,842,730	2.00×10^{-7}	9.86×10^{-3}	-	Intragenic
M1GA0008010	5	74,367,428	5.35×10^{-8}	2.64×10^{-3}	-	20,916	M1GA0021335	16	82,055,821	4.76×10^{-8}	2.35×10^{-3}	-	1,129,166
M1GA0008025	5	79,010,106	1.97×10^{-7}	9.75×10^{-3}	*SLC38A2*	1,029,163	M1GA0021462	16	84,330,710	1.87×10^{-7}	9.23×10^{-3}	*IRX4*	1,228,304
M1GA0008064	5	85,112,008	5.35×10^{-8}	2.64×10^{-3}	*LOC100738422*	51,201	M1GA0021563*	16	85,843,098	2.30×10^{-8}	1.14×10^{-3}	*LPCAT1*	5,430
M1GA0008091	5	91,724,948	1.97×10^{-7}	9.75×10^{-3}	*HAL*	769	ALGA0092903	17	5,999,136	5.10×10^{-9}	2.52×10^{-4}	*MTUS1*	53,654
M1GA0008099	5	92,805,456	1.18×10^{-7}	5.85×10^{-3}	-	1,025,329	M1GA0021675	17	20,631,316	9.80×10^{-8}	4.84×10^{-3}	*PLCB4*	Intragenic
M1GA0008133	5	100,729,561	1.97×10^{-7}	9.75×10^{-3}	*MGAT4C*	90,555	M1GA0021697	17	28,221,039	1.76×10^{-7}	8.72×10^{-3}	*BFSP1*	1,122,141
M1GA0008142	5	103,561,458	4.82×10^{-8}	2.38×10^{-3}	*U6*	92,710	ALGA0097816	18	32,645,078	7.79×10^{-8}	3.85×10^{-3}	*FOXP2*	1,020,806
M1GA0008159	5	105,349,192	2.60×10^{-8}	1.29×10^{-3}	*ACSS3*	Intragenic	ALGA0098168*	18	45,408,799	2.65×10^{-8}	1.31×10^{-3}	*GHRHR*	1,018,328
M1GA0008164	5	106,046,784	2.90×10^{-8}	1.43×10^{-3}	*OTOGL*	43,204	M1GA0008884	JH118434.1	15,0095	1.87×10^{-7}	9.23×10^{-3}	-	-
M1GA0008394	6	18,653,852	1.79×10^{-7}	8.83×10^{-3}	*CCDC135*	1,073,849	ALGA0030787	0	0	3.19×10^{-11}	1.57×10^{-6}	-	-
M1GA0008405	6	26,197,426	6.62×10^{-8}	3.27×10^{-3}	*LOC100517946*	80,522	ASGA0102104	0	0	1.94×10^{-9}	9.60×10^{-5}	-	-
M1GA0008418	6	27,367,862	6.71×10^{-8}	3.32×10^{-3}	*LOC100737013*	Intragenic	M1GA0006887	0	0	1.08×10^{-8}	5.32×10^{-4}	-	-
M1GA0008438	6	28,304,343	4.63×10^{-8}	2.29×10^{-3}	*TOX3*	1,002,255	M1GA0021138	0	0	2.90×10^{-8}	1.43×10^{-3}	-	-
M1GA0008473	6	30,900,258	4.76×10^{-8}	2.35×10^{-3}	*ZNF423*	36,367	ALGA0054046	0	0	9.71×10^{-8}	4.80×10^{-3}	-	-
ALGA0035254	6	40,760,438	1.61×10^{-10}	7.94×10^{-6}	*KIRREL2*	93,901							

[1]Single nucleotide polymorphisms with asterisk were associated with backfat thickness in our previous genomewide association study (Fontanesi et al., 2012d).

[2]P_{raw} = P nominal value.

[3]$P_{Bonferroni} = P < 0.01$ Bonferroni corrected.

[4]Additional information on close genes and Ensembl ID is reported in Supplementary Table 1.

[5]Single nucleotide polymorphisms not assigned to any Sscrofa10.2 position are indicated with chromosome (SSC) position "0." A few SNP were assigned to unassembled scaffolds. Several of these SNP were mapped on Sscofa9.2: ALGA0030787, SSC5, position 11,032,453; M1GA0006887, SSC4, 126,275,676; M1GA0021138, SSC16, 43,907,250; M1GA0011548, SSC8, 17,610; and ALGA0054046, SSC9, 46,348,451.

Other regions with 2 closely spaced (less than 1.5 Mb) SNP with $P_{Bonferroni} < 0.10$ were localized on SSC1 and SSC13 (Supplementary Table 1).

Functional Annotation of Associated SNP

Twenty-eight SNP with $P_{Bonferroni} < 0.10$ were in intragenic regions of recognized genes in the Ensembl Sscrofa10.2 assembly (Table 1; Supplementary Table 1). For the remaining mapped significant or suggestively significant SNP (number = 189), the distances from their closest genes ranged from 149 bp to 1.53 Mb (mean = 324.97 kb ± 22.33 kb, median = 22.48 kb).

Two or more SNP targeted the same gene. For example, 2 SNP (M1GA0006299, position 11,4151,582, and M1GA0006302, position 114,170,369) were located within or very close to the *immunoglobulin superfamily, member 3* (*IGSF3*) gene (Table 1) that is included in 1 of the regions with several significant or suggestively significant SNP on SSC4. This gene seems involved in immune cell regulation even if its function is not well characterized yet (Clark et al., 2001). Both markers were also associated with BFT in our previous study (Fontanesi et al., 2012d). Another 2 SNP on SSC4, included in another significant group of SNP of this chromosome (M1GA0006854, position 141,458,072, and M1GA0006869, position 141,552,372), were close and intragenic to the *heparan sulfate 2-O-sulfotransferase 1* (*HS2ST1*) gene, respectively. The *HS2ST1* gene encodes a member of the heparan sulfate biosynthetic enzyme family that transfers sulfate to the 2 position of the iduronic acid residue of heparan sulfate. This enzyme seems important in the signaling pathways involved in kidney formation and immunological functions (Muramatsu, 2000). Three SNP (M1GA0008164, position 106,046,784, DRGA0006447, position 107,303,857, and DRGA0006450, position 107,536,603), in 1 of the significant regions of SSC5, were upstream and downstream to the *otogelin-like* (*OTOGL*) gene. Mutations in this gene, which are mainly expressed in the inner ear of vertebrates during embryonic development, cause recessive deafness (Yariz et al., 2012). Its potential role on growth related metabolism or functions needs to be further investigated.

One of the most significant regions on SSC6 (26.20–30.90 Mb) might include the *fat mass and obesity associated* (*FTO*) gene that is associated with fat deposition traits in Italian Duroc, Italian Large White, and heavy pig commercial hybrids and feed conversion rate in Italian Large White (Fontanesi et al., 2009, 2010a; Fontanesi and Russo, 2013). To be precise, *FTO* position is available only on Sscrofa10.0 (27,697,754–28,086,339) as this gene is not assembled in Sscrofa10.2, but comparative mapping may confirm that its position on Sscrofa10.2 should be within the indicated region of SSC6 in the latest assembly (data not shown). This region also includes a marker associated with BFT (M1GA0008432; Fontanesi et al., 2012d).

The *LOC100157526* (also identified as *MYLK4- putative myosin light chain kinase 3-like*) and the *mitochondrial calcium uniporter regulator 1* (*MCUR1* or *CCDC90A*) gene, both located on SSC7, were each identified with 1 upstream and 1 downstream close marker (Supplementary Table 1). As far as we know, *MYLK4* is not functionally characterized in any species, yet. *MCUR1* encodes a component of mitochondrial Ca^{2+} uptake that regulates cellular metabolism (Mallilankaraman et al., 2012).

Highly significant SNP were close to additional genes. The most significant SNP on SSC1 (ALGA0004718, associated with BFT, and ALGA0004837) were close to a novel pseudogene and the *5-hydroxytryptamine* (*serotonin*) *receptor 1B*, *G protein-coupled* (*HTR1B*) gene. *HTR1B* is highly expressed in the brain and is associated with several behavior and neurological related functional roles. In dairy cattle, it is involved in the homeostatic regulation of lactation (Collier et al., 2012). M1GA0006343, one of the most significant SNP (also associated with BFT in our previous GWAS; Fontanesi et al., 2012d), located on SSC4, was close to the *olfactomedin-like 3* (*OLFML3*) gene that in pig may affect prenatal skeletal muscle development (Zhao et al., 2012). Another highly significant SNP of SSC4 (M1GA0006613) is close to the *Rho GTPase activating protein 29* (*ARHGAP29*) gene that is involved in cell spreading and endothelial barrier function, important in chronic inflammation, atherosclerosis, and vascular leakage (Post et al., 2013). A highly significant SNP on SSC6 (ALGA0035254) is close to the *kin of IRRE like 2* (*Drosophila*; *KIRREL2*) gene that encodes a cell adhesion molecule regulating neural activity-dependent formation of precise axonal projections in the main olfactory system (Serizawa et al., 2006). The highly significant SNP identified on SSC16 (M1GA0021128) was close to the putative *tetratricopeptide repeat protein 1-like* gene (LOC100519063) whose function is not characterized yet.

Several other genes have been tagged by the remaining SNP (Table 1; Supplementary Table 1). Therefore, to have a global picture of the potential functional role of regions around associated or suggestively associated SNP with ADG in our pig population, we used GO information of their corresponding closest genes and reported GO terms enriched in this dataset (Supplementary Tables 2 and 3). Thirty-five GO terms (Supplementary Table 2) and 31 annotation clusters (Supplementary Table 3) were retrieved. Several GO terms were significantly enriched if we considered a *P* nominal value: the 4 most significant terms ($P_{raw} < 0.02$) were 0005509 (calcium ion binding), 0045934 (negative regulation of nucleobase, nucleoside, nucleotide,

and nucleic acid metabolic process), 0051172 (negative regulation of nitrogen compound metabolic process), and 0051172 (negative regulation of nitrogen compound metabolic process), which might indicate a direct role of genes involved in several metabolic processes. However, none of the terms were significant after Bonferroni correction. This might suggest that, as expected, many different processes at different levels are involved in affecting this complex phenotype that expresses growth efficiency.

Comparison with Other Studies in Pigs

A few other GWAS for ADG or correlated production traits have been performed in other pig populations/breeds. Becker et al. (2013) performed a GWAS in a relatively small population of Swiss Large White boars for a large number of EBV for different traits but no significant markers have been reported for ADG. Sahana et al. (2013) performed a GWAS for feed efficiency in a Duroc population using 2 statistical approaches and identified a total of 79 and 44 significant SNP, respectively. The most significant markers were located on SSC4, SSC7, SSC8, and SSC14. None of the significant SNP that Sahana et al. (2013) reported in their study was significant in our GWAS for ADG even if several markers they identified were close (<0.5 Mb) to significant markers we reported on different chromosomes (e.g., SSC4, SSC5, SSC7, SSC16, and SSC17). Not overlapping results between Sahana et al. (2013) and our GWAS could be due to the different populations used and by the fact that the considered traits, even if correlated, are not the same. Another GWAS on residual feed intake (**RFI**) and other related traits (including ADG) was performed in purebred Yorkshires of 2 selection lines for RFI (high and low) using different approaches (Onteru et al., 2013). Significant SNP for RFI were identified on SSC3, SSC5, SSC6, SSC7, SSC13, and SSC14. Significant regions for ADG were reported in 15 SSC for a total of 44 chromosome positions. A few of these positions (SSC1, 167.00–168.00 Mb; SSC10, 15.00–16.00 Mb; SSC13, 36.00–37.00 Mb; and SSC16, 59.00–60.00 Mb) were very close to or included in the SNP list identified in this study. However, in general, results obtained by Onteru et al. (2013) poorly overlapped our results. This could be due to different experimental designs, incomplete power in the 2 studies, and/or differences between the investigated populations as already discussed comparing GWAS results for BFT in Italian Large White and other studies for the same trait (Fontanesi et al., 2012b).

The most significant region for ADG identified by Onteru et al. (2013) was on SSC1 and included the *MC4R* gene. A missense mutation in this gene (p.Asp298Asn) has been associated with several production traits including ADG in different pig populations (Kim et al., 2000) as well as in Italian Large White (Fontanesi et al., 2013). However, markers of the PorcineSNP60 BeadChip in the *MC4R* region were not significant in our GWAS for ADG, even if a few SNP had $P < 0.001$. This might indicate that, despite their effects, polymorphisms in the *MC4R* gene are not the most important markers to explain the variability of the target trait: their *P*-value could not pass the stringent threshold for significance we adopted in GWAS (Bonferroni corrected) that in single marker tests for a candidate gene is usually less stringent. Similar results for the *MC4R* region of SSC1 were obtained in our previous GWAS for BFT (Fontanesi et al., 2012d).

Other studies we performed in Italian Large White pigs showed a very strong effect of the *IGF2* intron3-g.3072G > A mutation (Van Laere et al., 2003) on ADG (Fontanesi et al., 2010b,c). Unfortunately, this gene is not assembled in Sscrofa10.2 and it was impossible to obtain a direct comparison with results obtained for SNP mapped on SSC2, which might be close to *IGF2*, included in the Illumina PorcineSNP60 BeadChip. We recently investigated another gene on SSC2 (*LDHA*) that was associated with ADG in the Italian Large White breed (Fontanesi et al., 2012b). This gene is localized at position 43898277 to 43909456 in the Sscrofa10.2 genome version. Markers in this region were not significant after Bonferroni correction but several had $P_{raw} < 0.01$; for example, ASGA0010122 (position 43911957) had a $P_{raw} = 0.0023$. A similar situation can be seen for the *MUC4* g.8227C > G polymorphism that we recently investigated. This gene is located on SSC13 (position 143786443–143842402) that we recently investigated. The g.8227C > G SNP, associated with susceptibility to enterotoxigenic *Escherichia coli* K88 strains (locus *F4bcR*), was antagonistically associated with ADG in Italian Large White and in Italian Landrace (Fontanesi et al., 2012a). In the current GWAS for ADG a marker close to this gene (ALGA0072062, position 143866440) had a $P_{raw} = 2.24 \times 10^{-5}$ that, however, could not pass the threshold for significance.

It seems that chromosome regions with moderate effects could not be detected in our GWAS for a few reasons: 1) high stringency of the significant threshold needed to overcome the problem of multiple testing, 2) linkage disequilibrium structure of the investigated population that could not be captured completely by the Illumina PorcineSNP60 BeadChip(L. Fontanesi, personal communication), and 3) the incomplete power of the experimental design, despite the adopted selective genotyping strategy tended to maximize it (Darvasi and Soller, 1992).

Implications

In this study, the genomewide association between DNA markers and ADG was analyzed in the Italian heavy pig breed for the first time. The investigated trait is includ-

ed in the selection index for the Italian Large White breed. The obtained results may contribute to understand the genetic mechanisms affecting ADG opening potential new perspectives to improve selection efficiency in this breed.

The study was designed to take advantage from the large number of pigs that are performance tested and genetically evaluated within the national selection program for this breed using a selective genotyping approach. Only extreme and divergent gilts for ADG EBV were genotyped to reduce the genotyping cost without losing much power (Darvasi and Soller, 1992; Van Gestel et al., 2000; Zhang et al., 2006). On the whole 229 SNP spread on all autosomes and on SSCX were significant (number = 127) or suggestively significant (number = 102). This large number of identified SNP might indicate that, according to the classical definition of a quantitative trait, a large number of genes, each with a small or medium effect, contributes to explain the genetic variability of ADG. This study also missed detecting some chromosome regions that might have a low/moderate effect on the target trait or other important regions probably due to features of the genotyping tool and assembled genome available. It is interesting to point out that about 1/10 (23/229) of SNP identified in this study were also associated with BFT in our previous GWAS (Fontanesi et al., 2012d), according to the high negative correlation between the 2 EBV in the Italian Large White population. These results might indirectly provide evidence on the correctness of the statistical approaches and the efficiency of the experimental designs we used in the 2 GWAS. Finally, the large number of genes and biological processes that should be involved in defining ADG indicates the complexity of the genetic factors affecting this ultimate phenotype. To better understand the biological mechanisms determining growth efficiency in pigs it will be important to dissect this phenotype into several intermediate and internal phenotypes.

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