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Exploiting single-marker and haplotype-based genome-wide association studies to identify QTL for the number of teats in Italian Duroc pigs

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Short title: **Genome scans for the number of teats in Italian Duroc pigs**

Abstract

The number of teats is a morphological trait of high economic relevance for the pig industry. Here, to dissect the genomic architecture of this trait in the Italian Duroc pig population, we present the results of genome-wide association studies in this Italian heavy pig breed. A total of 1,162 pigs, for which the number of teats was recorded, was genotyped with two high-throughput single nucleotide polymorphism (SNP) genotyping platforms (60K and 70K). Genome-wide association analyses were based on a single-marker approach and on a haplotype-based approach. Two quantitative trait loci (QTL) affecting the number of teats were identified. The most significant QTL, identified by the single-marker analysis and confirmed by the haplotype-based method, was located on *Sus scrofa* chromosome (SSC) 7, in the region of the *vertnin* (*VRTN)* gene. Suggestively associated markers (SNPs and haplotypes) were located on SSC10, in the region of the *FERM domain containing 4A* (*FRMD4A*) gene, the second identified QTL. These findings confirm previous results obtained in a few other Duroc populations. Overall, this study further supported the important role of variability in the *VRTN* gene region in affecting the number of teats in pigs. Moreover, the results also indicated that this trait in the Italian Duroc breed, as in many other pig breeds, is affected by few QTL, with the contribution of many other genetic factors with small effects, following the classical theory of quantitative traits.

Keywords: Breed: GWAS; heavy pig; single nucleotide polymorphism; *Sus scrofa*

1. Introduction

The number of teats is one of the most relevant morphological traits related to the reproductive performance of the sows. This trait is indirectly associated with the mothering ability, that, in turn, determines the number of piglets weaned per sow and per year (Kim et al., 2005; Andersen et al., 2011). To maximize the number of weaned piglets, selection programs, that have as main objective an increased litter size (a trait with low heritability), need to select, in parallel, for increased number of functional teats. Selection to improve the number of teats is, to some extent, facilitated by the medium to high heritability of the trait itself, as demonstrated by most studies in several pig breeds or lines that estimated the fraction of the genetic variance over the phenotypic variance for this morphological feature (e.g., Willham and Whatley 1962; Toro et al., 1986; McKay and Rahnefeld 1990; Borchers et al., 2002; Chalkias et al., 2013; Felleki and Lundeheim 2015; Balzani et al., 2016).

A broad variability in the number of teats (which could range from about 8 to 21) has been observed both across and within pig breeds and lines (Borchers et al., 2002; Lopes et al., 2014; Verardo et al., 2016; Rohrer and Nonneman 2017; Dall'Olio et al., 2018; van Son et al., 2019). Despite being represented by discrete and countable values, the number of teats is considered a quantitative trait determined by the effect of a high number of genetic factors. Several studies have however identified a few quantitative trait loci (QTL) affecting this morphological trait in pigs. The first QTL investigations were based on reference populations (F2 and backcrosses) constructed using parental animals of different breeds or lines (some of which with divergent number of teats), including some hyper-prolific Chinese breeds (e.g., Wada et al., 2000; Hirooka et al., 2001; Rodríguez et al., 2005; Bidanel et al., 2008; Ding et al., 2009; Hernandez et al., 2014). Subsequent studies based on high density single nucleotide polymorphism (SNP)

genotyping data explored the within breed variability via genome-wide association studies (GWAS). These studies confirmed the previously reported QTL regions and/or identified other novel genome regions affecting this trait (e.g., Arakawa et al., 2015; Rohrer and Nonneman 2017; Tang et al., 2017; Lee et al., 2019; van Son et al., 2019). One of the most relevant QTL for the number of teats, confirmed by several studies, is located on *Sus scrofa* chromosome (SSC) 7 (Mikawa et al., 2007, 2011; Duijvesteijn et al., 2014; Rohrer and Nonneman 2017; Dall'Olio et al., 2018; van Son et al., 2019; Moscatelli et al., 2020). At this QTL, alleles segregating in many populations are determined by variability in the *vertnin* gene, also known as *vertebrae development associate*d gene (*VRTN*; Mikawa et al., 2011; Arakawa et al., 2015). Originally reported to have pleiotropic effects on the number of vertebrae (Mikawa *et al.*, 2011), *VRTN* encodes a novel DNA-binding transcription factor which regulates the transcription of a set of genes that harbor VRTN binding motifs and modulates somite segmentation via the Notch signaling pathway (Duan et al., 2018). Some studies in a few pig populations proposed that variability in other genes on SSC7 [*latent transforming growth factor binding protein 2* (*LTBP2*); *BRMS1 like transcriptional repressor* (*BRMS1L*); and *Fos proto-oncogene, AP-1 transcription factor subunit* (*FOS*)], close to the *VRTN* gene, are involved in affecting the number of teats and the number of vertebrae (Zhang et al., 2016; Park et al., 2018; Liu et al., 2020).

Genome-wide association studies are usually run with a model fitting one marker at a time. However, to further extract information at the genome-wide level, the use of haplotypes has been recommended to capture additional marker-phenotype associations that cannot be detected with the single-marker approach (Lorenz et al., 2010; Barendse 2011). We recently applied haplotype-based analyses in a few GWAS in pigs and demonstrated that it is possible to improve the identification of genomic regions affecting

a targeted phenotype, complementing and completing the results obtained via a single-marker approach (Bovo et al., 2019, 2020, 2021). The additional information that can be retrieved is population-dependent, as it is related to the level of linkage disequilibrium between QTL alleles and the genotyped markers, which may vary among breeds, lines and populations. An ascertainment bias derived by the SNP composition of the commercial genotyping panels used in the investigation is also another element that should be considered in this context.

We recently carried out GWAS for the number of teats in two heavy pig breeds, Italian Large White and Italian Landrace, and identified several genome regions affecting this trait, not overlapping between the two breeds (Moscatelli et al., 2020; Bovo et al., 2021). Only in Italian Large White, the *VRTN* gene region was associated with this trait whereas in Italian Landrace no significant effects of *VRTN* (or other markers of SSC7) were evidenced (Bovo et al., 2021). For the other heavy pig breed, Italian Duroc, included in the Italian selection program conducted by the Italian Pig Breeders Association (ANAS), only few information on the segregation of the *VRTN* gene alleles was reported thus far (Fontanesi et al., 2014a). This Duroc population is a close nucleus constituted in the 1990' that is under selection with the main aim to optimize the production of green legs transformed in Protected Designation of Origin (PDO) dry cured hams. Other purebred Duroc pig populations (e.g., from USA, Canada, China, Japan and The Netherlands) have been already investigated with GWAS to identify genome regions affecting the number of teats. The investigations involving these populations reported partially overlapping results in terms of QTL regions (Arakawa et al., 2015; Yang et al., 2016; Tan et al., 2017; van Son et al., 2019; Zhuang et al., 2020; Li et al., 2021).

In this study, we carried out two GWAS for the number of teats in Italian Duroc heavy pigs using single-marker and haplotype-based approaches. Obtained results were

compared with the QTL information for the same trait reported in other pure-bred Duroc populations and in the other two Italian heavy pig breeds.

2. Materials and methods

2.1. Animals

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. All animals were part of the routine Italian pig breeding program and were slaughtered in a commercial authorized abattoir following standard procedures. Animals were not raised or sampled for the purpose of this study. As no treatment was given to any animals, no ethical approval was needed according to the rules of the animal research ethics committee of the University of Bologna based on the Italian legislation, as reported in the "DECRETO LEGISLATIVO 4 marzo 2014, n. 26".

The animals included in this study were from the national selection program of heavy pig breeds that is run by ANAS. A total of 1,164 Italian Duroc pigs, born in the years 1996-2018, was investigated (727 males and 437 females). Animals were included in the sib-testing evaluation program run by ANAS, described in previous reports (Fontanesi et al., 2012, 2014b, 2015). The number of teats on these animals was routinely recorded by direct counting at the beginning of the testing period and animals having less than 12 teats were excluded from the herd book of this breed.

2.2. Genotyping and SNP quality

DNA was extracted by using the Wizard Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA) from blood samples routinely collected on all pigs

included in the national selection program. A total of 606 animals were genotyped with the GeneSeek 70K GGP Porcine BeadChip (which includes 68,516 SNPs) whereas the remaining 558 pigs were genotyped with the Illumina PorcineSNP60 BeadChip v.2 (which includes 61,565 SNPs). The two panels presented a total of 41,862 shared genomic positions. Genotyping followed standard procedures based on the supplier's recommendations. DNA markers shared between the two SNP platforms were used in the analysis. BLAST+ v.2.7.1 (Camacho et al., 2009) was used to map SNPs to the Sscrofa11.1 reference genome and markers assigned to more than one position or assigned to sex chromosomes were discarded. Genotypes were evaluated with PLINK v.1.09 (Chang et al., 2015). Individual pigs with a call rate >0.90 and SNPs with a call rate > 0.90, a minor allele frequency (MAF) > 0.01 and in Hardy-Weinberg equilibrium (P > 0.0001) were retained for further analyses. The final dataset counted 1,162 animals (727 males and 435 females) and 29,604 DNA markers.

2.3. Haplotype estimation

The software SHAPEIT v.2 (Delaneau et al., 2011) was used for genotype phasing considering: (i) a genomic window size of 2 Mb, (ii) an effective population size (Ne) estimated with SNeP v.1.1 (Barbato et al., 2015) and (iii) a chromosome specific recombination rate given by Tortereau et al., (2012). The R package GHap 1.2.2 (Utsunomiya et al., 2016) was further used to call haplotypes considering a genomic window of 400 kb with a sliding block of 100 kb (Veroneze et al., 2013). Each haplotype was treated as bi-allelic DNA variant (genotypes are: NN, NH and HH; H = haplotype allele and N = NULL = all other N alleles present in the haploblock. PLINK was used to filter out haplotypes having a MAF < 0.02. A total of 85590 haplotypes coming from 66,363 overlapping haploblocks were estimated.

2.4. Linear mixed model analyses

To assess the association between the DNA markers (SNP or haplotypes) and the number of teats we used a linear mixed effect model. An additive genetic model assuming a trend per copy of the minor allele that specify the dependency of the number of teats on genotype categories was implemented as follows:

$$\boldsymbol{y} = \boldsymbol{W\alpha} + \boldsymbol{x}\beta + \boldsymbol{g} + \boldsymbol{e} \qquad (1)$$

where $\boldsymbol{y}$ ($n \times 1$) is a vector containing the phenotype (the number of teats) for the nth animal, $\boldsymbol{W}$ ($n \times k$) is a covariate matrix with $k = 2$ (a column of 1s and a column for the sex of the animal) and $\boldsymbol{\alpha}$ is the k-dimensional vector of covariates effects, $\boldsymbol{x}$ ($n \times 1$) is the vector containing genotypes for the ith DNA marker (SNP or haplotype), β is the additive fixed effect of the ith DNA marker on the phenotype, $\mathbf{g} \sim N(\mathbf{0}, \sigma^2_g \mathbf{K})$ is a multivariate Gaussian polygenic effect, with covariance matrix proportional to the relatedness matrix $\mathbf{K}$ ($n \times n$) and $\mathbf{e} \sim N(\mathbf{0}, \sigma^2_e \mathbf{I})$ is a multivariate Gaussian vector of uncorrelated residuals. Polygenic effect and residuals represented the random effects. The assessment of the association between each DNA marker and the total number of teats was obtained by testing the null hypothesis $H_0: \beta = 0$. Significance was tested by using the Wald test. All the models were fitted with GEMMA v.0.98 (Zhou and Stephens 2012) after computing the relatedness matrices $\mathbf{K_1}$ and $\mathbf{K_2}$ as a centered genomic matrices, for SNPs and haplotypes, respectively. Details about the genomic matrix construction are given in the manual of GEMMA. Bonferroni correction was applied to account for multiple comparisons, considering the total number of DNA markers used in each genome scan and value of $\alpha = 0.05$. Markers presenting a $P < 5.5 \times 10^{-05}$ were considered suggestive for associations (Wellcome Trust Case Control Consortium 2007). The proportion of

variance in phenotype explained (PVE) by each QTL was calculated as reported by Shim et al., 2015.

SNPs and haplotypes that had the lowest *P* in chromosome regions separated by at least 5 Mb were considered as tag DNA markers. GEMMA was also used to estimate the genomic (chip) heritability (h^2_G). Genomic control inflation factor (λ_{GC}) was computed in R v.3.6.0 (R Core Team 2018). Quantile-quantile plots (QQplots) and the Miami plot were generated in R by using the *qqman* package (Turner 2018).

2.5. Haploblock analysis and genome annotation

HaploView v.4.2 (Barrett et al., 2011) was used to study the structure of the haplotypes and linkage disequilibrium of the most interesting QTL regions. Each QTL was annotated considering the protein coding genes spanning the region of ± 500 kb around the evaluated DNA marker. The Sscrofa11.1 NCBI's GFF file and Bedtools v.2.17.0 (Quinlan and Hall 2010) were used for this purpose.

3. Results

3.1. Descriptive statistics

The numbers of teats in this pig population ranged from 12 to 17. About 42% of the pigs had 12 teats (Figure 1) as this represents the lower limit considered for including an animal in the Herd Book of this Italian heavy pig breed (ANAS 2021). Detailed information on the number of pigs with different numbers of teats are reported in Table S1. On average, pigs had 12.95 ± 0.97 (mean ± standard deviation) teats.

Table 1 reports the number of pigs and DNA markers used in GWAS. Assessment of results via QQplots (Figure S1) and the genomic control inflation factors (Table 1) indicated a good control of the population stratification.

Genomic heritability estimated for the number of teats in Italian Duroc pigs was moderate, with the value that increased from the estimates based on single-marker to the value obtained with the haplotype information (Table 1). This suggested that the haplotype analysis might have captured an additional fraction of heritability, as already reported for the same trait in other pig populations (Bovo et al., 2020, 2021).

3.2. Single-marker and haplotype-based association studies

The top associated DNA markers (SNPs and haplotypes) for each QTL region are reported in Table 2, whereas Table S2 lists all the significant and suggestively associated markers. Miami plot including SNP and haplotype data is showed in Figure 2.

Three SNPs and one haplotype on SSC7 were significantly associated with the number of teats. The most significant SNP was MARC0038565 (rs80894106; $P = 8.58\times10^{-10}$), located on SSC7 at position 97,652,632 bp, in the region of the *VRTN* gene (located on SSC7:97.61-97.62). This marker had a MAF = 0.44 (allele A) and a negative regression coefficient (β = -0.29), indicating a decrease in number of teats while increasing the number of copies of the minor allele. Hence, the Italian Duroc population had a higher frequency of the favorable allele (allele G) at this SNP position. This marker had also the highest proportion of variance in phenotype explained (PVE% = 3.22). The other two significant markers were closely located to the mentioned SNP and had a medium-high linkage disequilibrium (r^2 = 0.64, Figure 3) with the most significant marker. In general, this region did not show a very high level of linkage disequilibrium spanning the genotyped markers (Figure 3).

The peak of association on SSC7 was also highlighted by the haplotype analysis (SSC7:97.6-97.8 Mb; haplotype GAG; $P = 2.51\times10^{-8}$), confirming the involvement of the *VRTN* gene region in affecting the number of teats in the Italian Duroc pig breed. The

DNA marker MARC0038565 was included in this haplotype [first position in the haplotype sequence AAG; Figure 3]. Hence, the haplotype GAG [higher frequent in the population with f(GAG) = 0.52; favorable allele] and marker MARC0038565 presented similar MAF and β values.

A suggestively associated chromosome region identified both using the single-marker and the haplotype-based analyses was detected in the region of the *FERM domain containing 4A* (*FRMD4A*) gene located on SSC10:47.3-48.1 Mb. The results for the most significant SNP in this region, located at position 47,947,214 bp (ASGA0090802; rs81309209; $P = 4.80\times10^{-06}$), were confirmed by the haplotype analysis ($P = 5.08\times10^{-06}$). Both regions (SSC7 and SSC10) overlapped with the two QTL regions already reported in a previous study to affect the number of teats in Duroc pigs (van Son et al., 2019).

4. Discussion

Duroc pig populations are well known to have, in general, lower reproduction performances than other cosmopolitan pig breeds, as they are usually selected to maximize production performances and meat quality traits for the use of Duroc boars in terminal crosses (e.g., Cameron 1990; Gaugler et al., 1984; Skorupski et al., 1996; Hoque et al., 2007: Alam et al., 2021). However, to maintain a sustainable breeding in the selection nuclei of this breed, it is also needed to improve reproduction efficiency starting from traits with medium-high heritability.

A lower number of teats has been reported in Duroc populations than in the other two main cosmopolitan breeds, Large White and Landrace, which usually exceed the threshold of 14 teats. As such, 14 is the lower limit that is applied by ANAS to register animals to the Italian Large White and Italian Landrace herd books. In a recent survey that we carried out, the mean number of teats in these two breeds was 14.88 ± 0.92

(standard deviation) and 14.77 ± 0.87, respectively (Bovo et al., 2021), that are larger than what we observed in Italian Duroc pigs (12.95 ± 0.97). This number is however larger than what was reported in other Duroc populations. For example, Zhuang et al., (2020) reported the mean number of 10.90 ± 1.16 and 10.92 ± 1.14 in Duroc populations derived from US and Canadian nuclei and Tan et al., (2017) reported an average value of 10.72 ± 1.72 in a Chinese Duroc population. It is worth to mention that our statistics are biased as, in Italy, pigs with less than 12 teats are not registered in the Italian Duroc herd book and are therefore not considered for the calculation of the population mean in our study. Similar mean values to what we reported in the Italian Duroc breed were reported in a Dutch Duroc line (12.93 ± 1.05; van Son et al., 2019) and in another Chinese Duroc herd (13.17 ± 1.12; Li et al., 2021). These results suggest that some heterogeneity exists in Duroc populations, probably due to different selection pressures that have been applied on this trait in nuclei of this cosmopolitan breed around the world. Genomic heritability that we estimated in the Italian Duroc breed was close to what we already reported in Italian Landrace pigs using both single-marker and haplotype-based approaches (h^2_G ± standard error: 0.25 ± 0.02 and 0.30 ± 0.03, respectively) and a little lower than what we estimated in Italian Large White pigs (0.30 ± 0.03 and 0.43 ± 0.04, respectively; Bovo et al., 2021). Genomic heritability estimates for the number of teats were also reported in other Duroc populations. The single-marker heritability in an US nucleus was 0.19 ± 0.02, in a Canadian population was 0.34 ± 0.03 (Zhuang et al., 2020) and in a Chinese nucleus was 0.29 ± 0.05 (Li et al., 2021). These other studies, however, did not report any estimation based on haplotypes. Genomic heritability estimated using haplotype information indicated that this approach can potentially capture additional fractions of the so called “missed heritability” that would not be disclosed using single-marker approaches (Bovo et al., 2021). Therefore, the use of haplotypes might be useful to further

exploit genomic information for this purpose (Lorenz et al., 2010; Barendse 2011).

Only two QTL for the number of teats, one highlighted by significant markers and one identified by suggestively significant markers, were identified in the Italian Duroc population. Results obtained by the single-marker analysis were confirmed by the haplotype-based analysis. To continue the comparison with the results obtained in other Duroc populations, it is worth to mention that a limited number of segregating QTL for this trait has been also reported in other Duroc populations (Tan et al., 2017; van Son et al., 2019; Zhuang et al., 2020; Li et al., 2021). For example, by investigating a Dutch Duroc population, van Son et al., (2019) reported only the same two QTL regions that we identified in the Italian Duroc pigs. The larger number of pigs that was investigated in the Dutch study made it possible to reach significant results even for markers in the region of the *FRMD4A* gene that, however, remained less significantly associated than what was reported for the SNPs on SSC7, located in the region of *VRTN*. The *VRTN* region also included the most significant SNPs identified in GWAS carried out in Canadian Duroc pigs and in two Chinese Duroc populations (Tan et al., 2017; Zhuang et al., 2020; Li et al., 2021). No significant QTL for the number of teats were however identified in an American derived nucleus of Duroc pigs (Zhuang et al., 2020). Comparison with the results obtained in a Japanese Duroc population is not possible due to the different statistical approaches that were used to detect QTL regions for the number of teats (BayesC methods; Arakawa et al., 2015). The *VRTN* region, however, emerged as one of the most significant regions also in this population (Arakawa et al., 2015).

The proportion of variance in phenotype explained (PVE) by the most significant marker in the *VRTN* region that we identified in the Italian Duroc population was about half the PVE of the most significant marker of this region identified in a Dutch Duroc line (van Son et al., 2019): 3.2% *vs* 6.0%. This difference might be due to different

levels of linkage disequilibrium between the markers identified in these two Duroc populations (the Italian and the Dutch populations) and the causative mutation(s) and to the different experimental power determined by the number of animals analysed in our study and by van Son et al. (2019). Markers in this region have a much higher PVE for vertebral numbers are directly related phenotypes (van Son et al., 2019), which are probably the primary traits affected by *VRTN* variability (Mikawa et al., 2011), with pleiotropic effects on teat number.

A QTL pattern similar to what was identified in Italian Duroc pigs was already reported in Italian Large White pigs where, in addition to the *VRTN* and *FRMD4A* gene regions, only another significant haplotype region was identified on SSC12 (Bovo et al., 2021). The Italian Landrace population was however completely different, in terms of QTL regions for the number of teats, from both the Italian Duroc and Italian Large White breeds (Bovo et al., 2021).

These comparative analyses, in general, showed that one major QTL for the number of teats segregates in the most important cosmopolitan pig breeds and derived lines and populations (like the Italian Duroc breed). This QTL is located on SSC7 for which the most plausible causative mutation has been reported in the *VRTN* gene (Mikawa et al., 2011). In Italian Duroc pigs, the frequency of the insertion allele at the *VRTN* gene suggested to be the putative favorable allele (allele Q; Fontanesi et al., 2014a), which might increase the number of vertebrae and the number of teats (Mikawa et al., 2011), is almost equal to the frequency of the positive allele of the most significant SNP that we identified on SSC7 (allele G of MARC0038565). This information could indirectly suggest a very high linkage disequilibrium between the two markers. Additional studies are needed to complete the linkage disequilibrium pattern of this SSC7 genome region in Italian Duroc, including genotyping data of the *VRTN* gene which might

confirm the significant results obtained for the MARC0038565 SNP. Additional studies are also needed to characterize the variability in the *FRMD4A* gene and identify the causative mutation(s) affecting this trait. This gene encodes a FERM domain-containing protein that regulates epithelial polarity by connecting ADP ribosylation factor 6 (ARF6), which is considered an important factor involved in actin cytoskeleton dynamics and membrane trafficking (Ikenouchi and Umeda 2010). Therefore, the role of this gene in affecting the number of teats is not obvious. Thus, it will be also important to clarify the molecular mechanisms that could lead to this effect and eventually evaluate the effect of other close genes in determining the QTL located on SSC10.

4. Conclusion

In terms of genotyped animals, this is the largest GWAS carried out in the Italian Duroc pig breed thus far. The estimated genomic heritability that we obtained confirmed that genetic factors affect this trait in a similar way as it was evidenced in other Duroc populations. The limited number of major QTL influencing this trait and segregating in the Italian Duroc breed indicates that a marker assisted selection based on *VRTN* alleles and eventually on *FRMD4A* gene markers, might be effective only in part as it would not completely exploit the genetic variance related to this trait for which many other genes are involved. Their effect could be captured including this trait in a genomic selection program that can take into account genome-wide variability.

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Table 1. Datasets used in the single-marker and haplotype-based genome-wide association studies carried out in Italian Duroc pigs.

Parameter	**Single-marker**	**Haplotype-based**
Animals used in GWAS (*n*)	1162	1162
Genotyped DNA markers used in GWAS (*n*)	29604	85590
Genomic heritability h^2_G (standard error)	0.25 (0.05)	0.30 (0.06)
Inflation factor (λ_{GC})	1.015	1.010

Table 2. Genomic regions associated and significantly associated with the number of teats in Italian Duroc pigs. Results are stratified by marker position.

Genome scan[1]	SSC[2]	Pos[3]	Marker[4]	Min/Maj[5]	MAF[6]	*B*[7]	*s.e.*[8]	*P*[9]	PVE(%)[10]	Candidate gene
Haplotype-based	7	97600001	CHR7_B920_GAG	N/H	0.480	-0.256	0.046	2.51×10^{-8}	2.60	*VRTN*
Single-marker	7	97652632	MARC0038565	A/G	0.442	-0.286	0.046	8.58×10^{-10}	3.22	*VRTN*
	7	98066911	H3GA0022664	A/G	0.442	-0.233	0.047	8.92×10^{-7}	2.07	*VRTN*
	7	98089286	ASGA0035527	G/A	0.442	-0.233	0.047	8.96×10^{-7}	2.07	*VRTN*
Haplotype-based	10	47000001	CHR10_B437_AGA	N/H	0.460	0.211	0.046	5.08×10^{-6}	1.78	*FRMD4A*
Single-marker	10	47947214	ASGA0090802	A/G	0.450	-0.209	0.045	4.80×10^{-6}	1.82	*FRMD4A*

[1] Genome scans performed in the Italian Duroc population.
[2] *Sus scrofa* chromosome.
[3] Position, in base pairs, on the *Sus scrofa* reference genome (version Sscrofa11.1).
[4] DNA marker identifier reported in the chip panel. For haplotypes, the haploblock identifier (chromosome specific) and the allele is reported.
[5] Minor/Major alleles. Haplotypes have been treated as bi-allelic variants (H = haplotype allele and N = other *N* alleles).
[6] Minor allele frequency.
[7] Regression coefficient. A positive value indicates that the no. of teats increases with the increasing of the number of copies of the minor allele. A negative value indicates that the no. of teats decreases with the increasing of the number of copies of the minor allele.
[8] Standard error of the regression coefficient.
[9] *P* at the Wald test of GEMMA.
[10] Proportion of variance in phenotype explained (%) by the DNA marker.

Figure 1. Distribution plot of the number of teats in the Italian Duroc population. The number of animals is reported at the top of each bar.

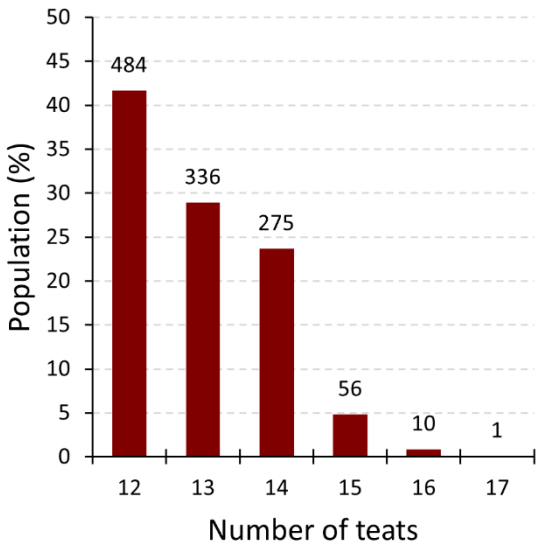


Figure 2. Miami plot of the number of teats in the Italian Duroc population. Results of the single-marker GWAS are on the top part of the plot whereas results of the haplotype-based analysis are on the bottom. Dots represent the single nucleotide polymorphism (SNP) or the haplotype markers. The red lines identify the significance thresholds (Bonferroni correction; $\alpha = 0.05$). Statistically associated SNPs are highlighted in green.

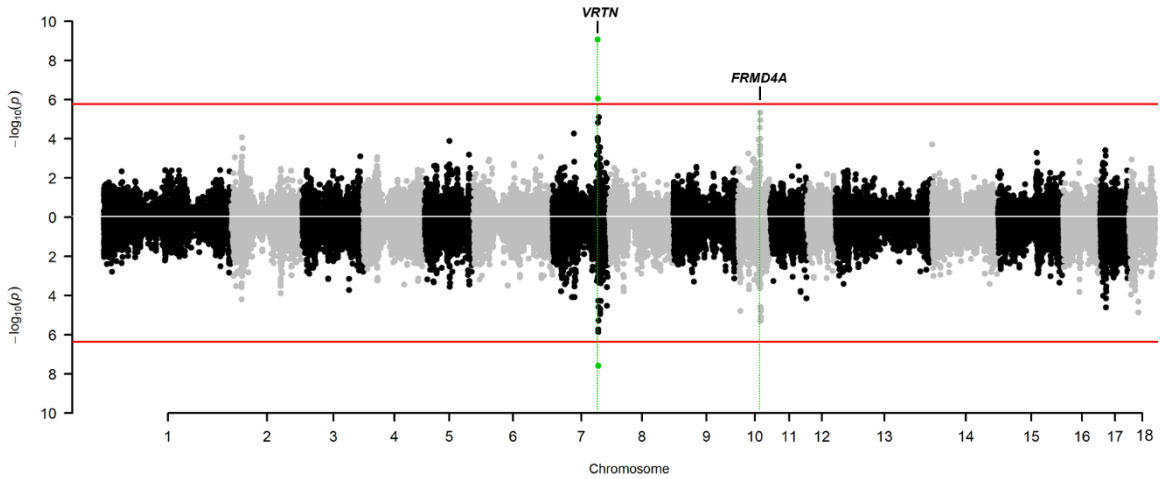
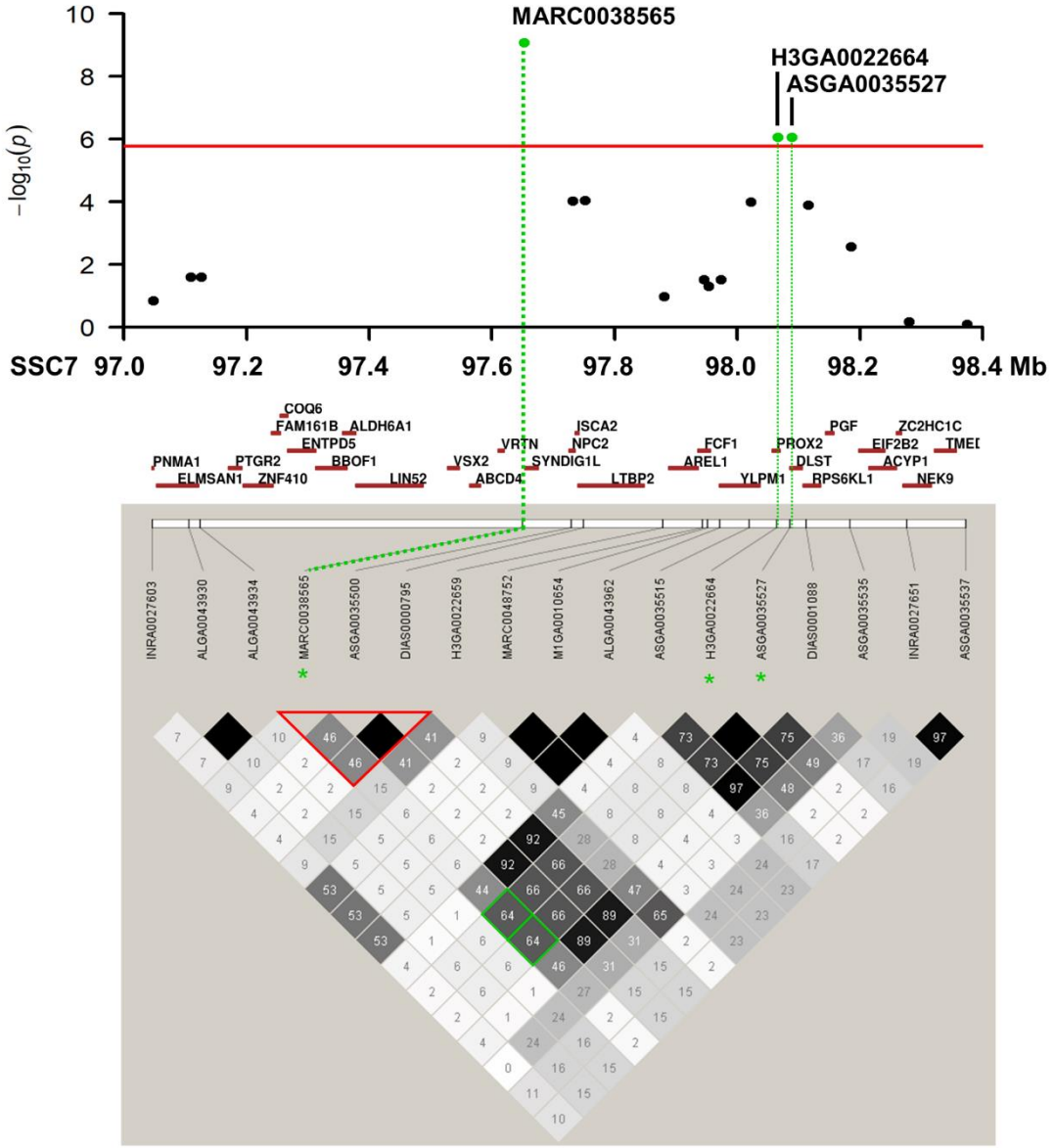


Figure 3. Regional association plot (single-marker analysis) for the SSC7:97.0-98.4 Mb genomic region. Linkage disequilibrium (LD) was measured between SNP pairs as r^2 and it is reported/showed in each box coloured in relation to its magnitude. The associated SNPs are marked with a green star symbol whereas DNA markers within the top associated haplotype (CHR7_B920_97400001_97800001_GAG) are marked with a red triangle.



Supplementary Material

Table S1. Distribution of the number of teats in the Italian Duroc pigs.

Teat number class	Total no. of pigs (%)[§]	No. of females (%)	No. of males (%)
12	484 (41.65)	177 (40.69)	307 (42.23)
13	336 (28.92)	130 (29.89)	206 (28.34)
14	275 (23.67)	106 (24.37)	169 (23.25)
15	56 (4.82)	20 (4.60)	36 (4.95)
16	10 (0.86)	2 (0.46)	8 (1.10)
17	1 (0.09)	0 (0)	1 (0.14)
TOTAL	1162	435	727

[§] Percentages are given within class (population, males, females).

Table S2. Genomic regions affecting the number of teats ($P < 5.5 \times 10^{-05}$) in Italian Duroc pigs. Results are sorted by chromosome and *P*.

Genome scan[1]	SSC[2]	Pos[3]	Marker[4]	Min/Maj[5]	MAF[6]	*β*[7]	*s.e.*[8]	*P*[9]
Single-marker	7	97652632	MARC0038565	A/G	0.44	-0.286	0.046	8.58E-10
Haplotype-based	7	97600001	CHR7_B920_97400001_97800001_GAG	N/H	0.48	-0.256	0.046	2.51E-08
Single-marker	7	98066911	H3GA0022664	A/G	0.43	-0.233	0.047	8.92E-07
Single-marker	7	98089286	ASGA0035527	G/A	0.43	-0.233	0.047	8.96E-07
Haplotype-based	7	97600001	CHR7_B920_97400001_97800001_AGA	H/N	0.33	-0.237	0.049	1.36E-06
Haplotype-based	7	97500001	CHR7_B919_97300001_97700001_AA	H/N	0.35	-0.231	0.048	1.84E-06
Haplotype-based	7	98600001	CHR7_B930_98400001_98800001_ACG	N/H	0.41	-0.210	0.046	5.23E-06
Single-marker	7	100073102	ALGA0044076	A/G	0.41	-0.220	0.049	7.96E-06
Haplotype-based	7	101900001	CHR7_B963_101700001_102100001_GAAAAGC	H/N	0.39	-0.211	0.048	1.13E-05
Haplotype-based	7	101800001	CHR7_B962_101600001_102000001_GGGAAAAG	H/N	0.39	-0.209	0.048	1.47E-05
Single-marker	7	98469335	ASGA0035543	G/A	0.41	-0.205	0.047	1.54E-05
Haplotype-based	7	101700001	CHR7_B961_101500001_101900001_AGAGGGGAAAA	H/N	0.39	-0.205	0.048	2.02E-05
Haplotype-based	7	98700001	CHR7_B931_98500001_98900001_GG	N/H	0.38	-0.211	0.050	2.53E-05
Haplotype-based	7	116400001	CHR7_B1106_116200001_116600001_AGAAGGA	H/N	0.04	0.424	0.101	2.93E-05
Single-marker	10	47947214	ASGA0090802	A/G	0.45	-0.209	0.045	4.80E-06
Haplotype-based	10	47000001	CHR10_B437_46800001_47200001_AGA	N/H	0.46	0.211	0.046	5.08E-06
Haplotype-based	10	47200001	CHR10_B439_47000001_47400001_AGAAAGGA	N/H	0.50	0.209	0.046	6.14E-06
Haplotype-based	10	47100001	CHR10_B438_46900001_47300001_AGAAAG	N/H	0.46	0.208	0.046	7.83E-06
Haplotype-based	10	47400001	CHR10_B441_47200001_47600001_AAGGAGGCA	N/H	0.49	0.212	0.047	8.37E-06
Single-marker	10	47963773	ASGA0094083	A/G	0.46	-0.201	0.046	1.12E-05
Haplotype-based	10	3200001	CHR10_B31_3000001_3400001_GAGGA	H/N	0.04	0.475	0.110	1.64E-05
Haplotype-based	10	47500001	CHR10_B442_47300001_47700001_GAGGCAGC	H/N	0.48	-0.201	0.046	1.65E-05
Haplotype-based	10	47600001	CHR10_B443_47400001_47800001_GGCAGCACG	H/N	0.49	-0.192	0.045	1.77E-05
Haplotype-based	10	47300001	CHR10_B440_47100001_47500001_AAAGGAG	N/H	0.49	0.201	0.047	1.79E-05
Haplotype-based	10	47700001	CHR10_B444_47500001_47900001_GCAGCACGG	H/N	0.46	-0.194	0.046	2.30E-05
Haplotype-based	10	46900001	CHR10_B436_46700001_47100001_AG	N/H	0.31	0.211	0.050	2.59E-05
Single-marker	10	47026618	ALGA0059028	G/A	0.31	0.209	0.050	2.80E-05

[1] Genome scans. SNPs and Haplotypes indicates which DNA markers have been used to carry out the genome scans.

[2] *Sus scrofa* chromosome.
[3] Position, in base pairs, on the *Sus scrofa* reference genome (Sscrofa11.1).
[4] DNA marker identifier reported in the chip panels. For haplotypes, we report; chromosome, haploblock, start, end, haplotype.
[5] Minor/Major alleles. Haplotypes have been treated as bi-allelic variants (H = haplotype allele and N = other *N* alleles).
[6] Minor allele frequency.
[7] Regression coefficient. A positive value indicates that the no. of teats increases with the increasing of the number of copies of the minor allele. A negative value indicates that the no. of teats decreases with the increasing of the number of copies of the minor allele.
[8] Standard error of the regression coefficient.
[9] *P* at the Wald test of GEMMA.

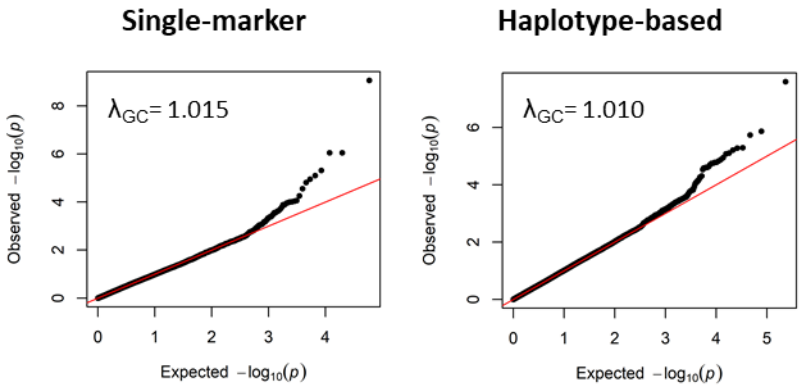



Figure S1. Quantile-quantile plots of the genome-wide association studies carried out in the Italian Duroc population. Inflation factor (λ_{GC}) is reported.