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# Genome-wide association study identifies markers associated with meat ultimate pH in Duroc pigs

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## Summary

In this study we aimed to identify genomic regions associated with muscle pH, meat colour and water-holding capacity in a population of 280 Italian Duroc pigs genotyped by the Illumina PorcineSNP60 v2 Genotyping BeadChip. After quality control, the remaining 32 597 SNPs and 278 subjects were used to perform a genome-wide association study with the GENABEL package, using a kinship matrix in a model with the effects of sex, age and slaughter day. Bonferroni correction was applied, and the significant markers and regions were then further investigated to identify the nearest genes and the linkage disequilibrium (LD) between markers. Four markers (ASGA0082344, ASGA0095635, DBWU0000985 and CASI0005117) were significantly associated with ultimate pH (pH<sub>U</sub>); no significant association was detected for the other traits. The four significant variants, located from 16.841 to 17.643 Mb on chromosome 3, were found within or close to the sequences of the sulfatase modifying factor 2 (SUMF2), lysine acetyltransferase 8 (KAT8), serine protease 8 (PRSS8) and phosphorylase kinase catalytic subunit gamma 2 (PHKG2) genes. The four associated markers lie in two LD blocks, suggesting that the observed effect is related to mutations located in two regions: the first one where SUMF2 is mapped and the second one where genes KAT8, PRSS8 and PHKG2 are located.

## Keywords

genetic markers, GWAS, meat quality traits, swine, pH<sub>U</sub>

Muscle pH, meat colour and water-holding capacity (WHC) are parameters that strongly influence pig products organoleptic quality and have a prominent economic value for the processing industry (Ciobanu et al. 2011). Among these traits, meat pH measured 24 h after slaughter (pH<sub>U</sub>) is one of the most important qualitative characteristics (Ciobanu et al. 2011), also influencing meat colour, WHC and the quality of dry-cured hams. Several candidate genes associated with meat pH, colour and WHC have already been reported, but to date the knowledge about the causal mutations affecting these traits is still lacking. This research was aimed at identifying through a genome-wide association study the markers and genes associated with muscle pH, meat colour and WHC in a population of 280 Italian Duroc pigs.

The purebred population provided by the Italian National Association of Pig Breeders (ANAS, <http://www.anas.it>) was reared in the same environmental conditions from 30 to about 155 kg of

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live weight. Animal care and slaughter were performed in compliance with the European rules [Council Regulation (EC) No. 1/2005 and Council Regulation (EC) No. 1099/2009]. All slaughter procedures were monitored by the veterinary team appointed by the Italian Ministry of Health. The Italian Duroc pigs were slaughtered at eight months of age in 20 slaughter days. A sample of semimembranosus muscle (SM) was collected from each animal and stored at -20 °C until DNA extraction.

The phenotypes measured for SM were: pH<sub>1</sub> (measured about 1 h post mortem), pH<sub>U</sub> (measured 24 h post mortem), CIE L\*a\*b\* colour (measured using Konica Minolta Chroma Meter CR-300; Konica Minolta Sensing, Inc.) and WHC (calculated using the filter paper press method; Hofmann et al. 1982).

Genomic DNA was extracted using the Genomic DNA Purification Kit (Promega Corp.). The samples were then genotyped using the Illumina PorcineSNP60 v2 Genotyping BeadChip.

The coordinates of the 61 565 SNPs were updated on *Sus scrofa* genome assembly Build 11.1. A total of 11 332 SNPs were not considered further because they were unmapped or located on sex chromosomes. After conducting quality control using the GENABEL package (Aulchenko et al. 2007), the remaining 278 individuals and 32 597 SNPs were used to calculate a kinship matrix, which was then employed in a model with sex, age and slaughter day effects. Bonferroni correction was applied, and the significant markers and genomic regions were further investigated using HAPLOVIEW (Barrett et al. 2005). The detailed workflow of the performed analyses is reported in **Table S1**.

Four markers associated with pH<sub>U</sub> passed the Bonferroni threshold of  $P = 1.53E-06$  (**Table 1, Fig. S1**). Manhattan plots for the other traits are reported in Fig. S2. For these phenotypes, we did not find markers reaching Bonferroni corrected significance levels. The four markers significantly linked to pH<sub>U</sub> are located from 16.841 to 17.643 Mb on *S. scrofa* chromosome (SSC) 3. The two markers with the highest associations (ASGA0082344 and ASGA0095635) belong to the sulfatas modifying factor 2 (SUMF2) gene, the SNP DBWU0000985 is located in the downstream region of lysine acetyltransferase 8 (KAT8) and in the 3<sup>0</sup> –untranslated region of serine protease 8 (PRSS8) and the marker CASI0005117 is an intron variant of the phosphorylase kinase catalytic subunit gamma 2 (PHKG2) gene.

All these markers and related genes are located in a 2-Mb DNA segment on SSC3 of a wider QTL region (QTL #4124, between 14.8 and 21.9 Mb) already reported in Pig QTLdb to be associated with SM pH<sub>U</sub> (de Koning et al. 2003). Interestingly, the four significant markers lie in two linkage disequilibrium (LD) blocks (Fig. 1): block 2, comprising ASGA0082344 and ASGA0095635, and block 3, harbouring DBWU0000985 and CASI0005117. It is possible to presume that the observed effect could be due to mutations in one or both these regions: the first one where SUMF2 is mapped and the second one where KAT8, PRSS8 and PHKG2 are located. The list of genes included in these two linkage blocks is reported in **Table S2**.

Moreover, it is worth noting that the SUMF2 gene partly overlaps the phosphorylase kinase catalytic subunit gamma 1 (PHKG1), a gene coding for a subunit of the phosphorylase kinase (PhK) enzyme. This enzyme is a 16-subunit protein kinase complex, and both PHKG1 and PHKG2 code for two out of the 16 subunits. PhK is involved in glycogenolysis, and PHKG1 mutations were already reported to affect muscle PhK activity, glycolytic potential and pH in Suta pigs (Ma et al. 2014). Our results confirm the importance of the SSC3 region between 16 and 18 Mb on porcine SM pH<sub>U</sub> and suggest that a more detailed analysis of this region could be useful to investigate the genetic regulation of the pH<sub>U</sub> trait.

**Table 1** Significant markers identified for pH<sub>U</sub>, with their location, adjusted P-value and SNP position relative to the nearest gene.

SNP (variant ID)	SSC <sup>1</sup>	Location (bp) <sup>2</sup>	P-value <sup>3</sup>	Nearest gene <sup>4</sup>	SNP position relative to the nearest gene <sup>5</sup>
ASGA0082344 (rs81330887)	3	16 841 101	7.57E-07	SUMF2	Exon variant (exon 2, type: synonymous); 5' UTR variant
ASGA0095635 (rs81315085)	3	16 842 436	7.57E-07	SUMF2	Intron variant
DBWU0000985 (rs55618510)	3	17 362 365	7.84E-07	KAT8; PRSS8	Downstream variant; 3' UTR variant
CASI0005117 (rs321892564)	3	17 642 214	1.16E-06	PHKG2	Intron variant

UTR, untranslated region.

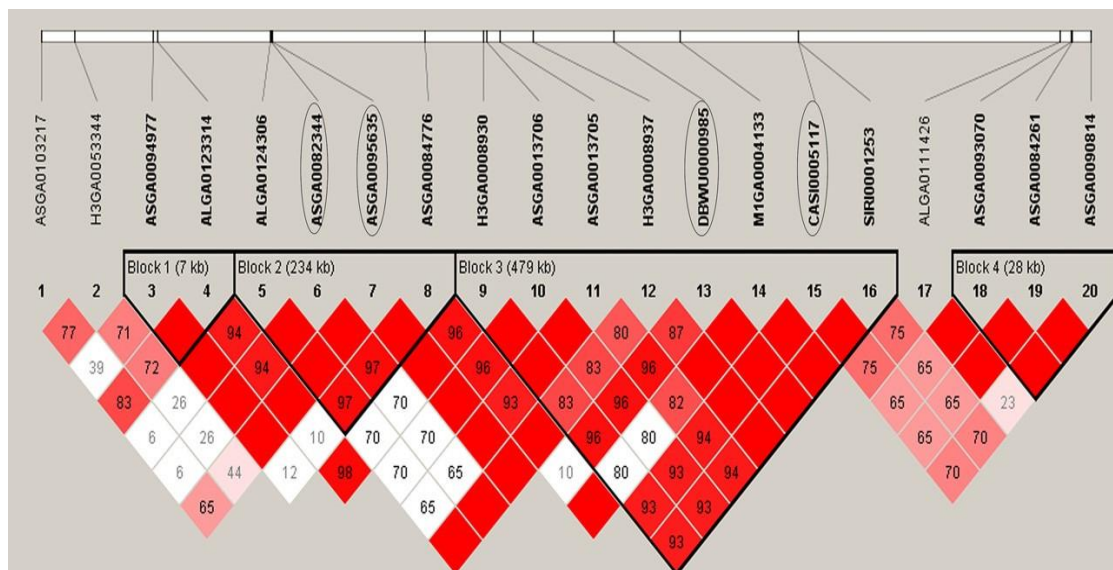
<sup>1</sup>Sus scrofa chromosome.

<sup>2</sup>SNP positions referred to S. scrofa assembly Build 11.1, expressed in bp.

<sup>3</sup>Bonferroni corrected P-value.

<sup>4</sup>SNP nearest gene named with official gene symbol.

<sup>5</sup>SNP position relative to the nearest gene or genes.



**Figure 1** SSC3 linkage blocks where the markers associated with pH<sub>U</sub> trait are located. SNPs in circles are the significant markers detected by GWAS.

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<https://www.animalgenome.org/repository/pub/ITALY2018.0807/>

## Conflict of interests

The authors declare that they have no conflict of interests.

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## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1** (a) Quantile–quantile plots of observed vs. expected distribution of P-values for the genome-wide association study (GWAS) of pH<sub>U</sub> trait. (b) Manhattan plot showing the GWAS significance for the associations between the SNPs and the pH<sub>U</sub> trait.

**Figure S2** Manhattan plot of genome-wide association study results for pH<sub>1</sub>, CIE-L\*, CIE-a\*, CIE-b\* and WHC traits. Table S1 Complete workflow of the genome-wide association study analyses.

**Table S2** List of genes mapped in the SSC3 region between 16.8 and 17.7 Mb.