# In silico analysis of meat quality candidate genes among Nero Siciliano, and Italian heavy pigs genomes

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## SUMMARY

In a modern context, where consumers are becoming better educated and oriented towards native animal breeds including meat with a high nutritional profile, the autochthonous pig breeds represent an important genetic reserve to be utilized mainly for the production of typical Italian products.
Autochthonous pig breeds represent a valuable genetic reserve to be utilized for typical products linked to italian gastronomic traditions, or for recovering some organoleptic proprieties of pork which have been lost through of severe selective programmes. This can be a strong stimulus for the conservation of local breeds.
The Food and Agriculture Organization (FAO) has expressed concern about the lack of interest in local breeds compared to high-output animals and conservation programs have been implemented by various countries worldwide.
In this study we report an in silico comparison of 48 candidate genes involved in meat quality traits in pig. The genes were analyzed from different pig breeds [Nero Siciliano (NS), Large White (LW), Landrace (LAN) and Duroc (DU)] whose genomes were previously sequenced and published.
In particular, we focused on genes related to muscle mass deposition and carcass fatness as these traits influence technological processes adopted for long matured pork meats products such as cured ham. More than twenty thousand variants were identified by comparing the gene set of each breed to the reference genome assembly. Of these ~22,000 were SNPs, ~3,000 short insertions and ~1400 short deletions. Transitions/transversions ratio was 2,650 while missense/silent ratio resulting in 0.526. Furthermore, over 40% of intronic variants and ~45% of non coding transcript variants were also identified. Among all variants detected in this study, more than 3,000 were shared among NS, LW and LAN while ~7,000 were unique for NS, ~2,000 for LW and ~6000 for LAN, showing a high degree of genetic variability among studied breeds. This study represents a first preliminary study of genetic characterization of Nero Siciliano pig and also provides a platform for future comparative studies between this and other swine breeds.

## KEY WORDS

Nero Siciliano pig, fatness gene, *in silico* analysis, unique variants, meat quality traits.

## INTRODUCTION

Currently, the survival and the assessment of local breeds is linked to various factors such as their rusticity, adaptation to difficult environmental conditions and a higher market value of their products compared to industrial ones. In Italy, and more generally in Europe, the trend is no longer to produce more quantities of food but to move towards the assurance of high quality products, an attitude that could be a strong stimulus for the conservation of local breeds through the production of typical products[1].
Autochthonous pig breeds represent a valuable genetic resource to utilise for typical products linked to the gastronomic traditions of Italy, or for recovering some organoleptic proprieties of pork actually lost because of severe selective programmes[2].

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In the last few years, many local breeds have been subjected to genetic erosion and loss of biodiversity resulting in the impoverishment of a precious gene pool that has mainly affected marginal areas and low input breeding systems. Nero Siciliano (NS) pig is a local breed reared under semi-extensive system mainly in the Nebrodi mountains of Sicily (Italy)[3]. In 2003 was established a consortium for the valorization of Sicilian meat productions and a request to recognize the fresh Nero Siciliano meat with the Protected Denomination of Origin (PDO) was issued in 2005. In addition, an official request of PDO was also initiated for Nero Sicliano's cured ham in 2011.
The genetic variability of the Nero Siciliano pig has been assessed with the use of various genetic markers in several studies focusing on molecular characterization and genetic structure of coat colour genes (*MC1R* and *KIT* gene) in order to evaluate their usefulness for breed traceability[4].
Although in the last years admirable efforts have been made to recover the extremely threatened biodiversity of pigs, today only few Italian local breeds are able to withstand the

competition with foreign commercial breeds (i.e. the Large White, Landrace and Duroc) and/or with other commercial crossbreeds today widespread in the market.

Fat deposition is a trait extensively studied in pigs due to its implications on the efficiency of animal's growth and on the technological and nutritional characteristics of meat products, with significant implications on business management[5]. An important goal of pig selection program in Italy is to obtain animals with a high aptitude for dry-cured ham production, such as Parma or S. Daniele hams.

In the last few years, the genomes of different pig breeds have been sequenced and release in public freely accessible databases and therefore the efficiency of the selection process could be improved by the implementation of molecular data into breeding programs. For this reason, in this study, we analyzed 48 candidate genes involved in meat quality traits in 4 different pig breeds including the Nero Siciliano, whose genome sequence has also been recently determined.

## MATERIALS AND METHODS

In this study, the *Sus scrofa 11.1* (DU) reference genome (Genbank: GCA_000003025.6) was used in the comparative analysis. The reads from the other pig breeds were retrieved from the NCBI Sequence Read Archive (SRA) database LW (SRR3123346; SRR3123347), LAN (ERX2292210), NS (SRX3406507). All the reads were initially checked with the FastQC program, cleaned with Trimmomatic v. 0.36,[6] and then mapped against the reference genome using the BWA aligner for variant calling analysis[7].

The reads of NS were obtained by our recent genome sequencing project[8] and used for the genome-wide analysis carried out to identify potentially breed-related genetic variants. Briefly, for NS genome sequencing, genomic DNA (gDNA) was extracted from a blood sample of a male NS pig using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Italy) and quantified by Qubit 2.0 fluorometer with Qubit dsDNA HS Assay Kit (Thermo Fisher, Italy). PCR-Free library preparation was performed using the TruSeq DNA kit (insert size 350 bp) and 1 g of gDNA, following the protocol provided by Illumina. Then, paired-sequencing was carried out using the HiSeqX platform (Illumina®). SNPs and short INDELs discovery in 48 genes related to lipid metabolism and fat deposition was performed using SUPER-CAP program and the potential effects of resulting mutations were evaluated by SnpEff software (v4_3m_core)[9]. Furthermore, unshared SNPs of NS were analyzed with VEP tool (www.ensembl.org) in order to evaluate novel and existing variants and to annotate them. These genes were chosen because they affect phenotypes related the meat quality and fatness traits.

**Table 1** - List of 48 fatness related genes investigated in this study. The table show the Chromosome, Gene Symbol, Sequence name, Starting and ending coordinates.

| Gene | N° Variants (SNPs/short INDEL) | Seq_name | Chr | Start | End |
|---|---|---|---|---|---|
| IGF2 | 95 | CM000813.5 | 2 | 1469183 | 1496417 |
| LEP | 222 | CM000829.5 | 18 | 20106867 | 20124071 |
| LEPR | 1028 | CM000817.5 | 6 | 146802297 | 146896152 |
| MC4R | 1 | CM000812.5 | 1 | 160772013 | 160774124 |
| PIK3C3 | 1448 | CM000817.5 | 6 | 125890598 | 126038753 |
| CTSB | 221 | CM000825.5 | 14 | 15014139 | 15035081 |
| CTSD | 75 | CM000813.5 | 2 | 1188548 | 1197642 |
| TRIB3 | 95 | CM000828.5 | 17 | 34808273 | 34819592 |
| PCSK1 | 152 | CM000813.5 | 2 | 103004794 | 103050707 |
| MUC4 | 135 | CM000824.5 | 13 | 134192412 | 134248435 |
| FTO | 2369 | CM000817.5 | 6 | 31177112 | 31564674 |
| TBC1D1 | 2986 | CM000819.5 | 8 | 29370344 | 29590574 |
| PDE1C | 3536 | CM000829.5 | 18 | 40818316 | 41410555 |
| CRISP1 | 416 | CM000818.5 | 7 | 43883536 | 43911877 |
| STAT4 | 375 | CM000826.5 | 15 | 95656206 | 95764099 |
| GPR120 | 72 | CM000825.5 | 14 | 105011408 | 105033633 |
| CSTB | 11 | CM000824.5 | 13 | 206706060 | 206710598 |
| SCPEP1 | 558 | CM000823.5 | 12 | 33265509 | 33296805 |
| SCD | 66 | CM000825.5 | 14 | 111461560 | 111478033 |
| FASN | 55 | CM000823.5 | 12 | 922405 | 937560 |
| LIPE | 31 | CM000817.5 | 6 | 49543671 | 49560126 |
| PLIN1 | 35 | CM000818.5 | 7 | 55237517 | 55250797 |
| PLIN2 | 139 | CM000812.5 | 1 | 203683867 | 203709277 |
| CA3 | 7 | CM000815.5 | 4 | 51227485 | 51237861 |
| DECR1 | 179 | CM000815.5 | 4 | 46731359 | 46767427 |
| STAB1 | 84 | CM000824.5 | 13 | 34630448 | 34659371 |
| IDH3B | 27 | CM000828.5 | 17 | 32955061 | 32960110 |
| FYB | 1900 | CM000827.5 | 16 | 24332539 | 24507768 |
| FABP3 | 33 | CM000817.5 | 6 | 87943003 | 87950708 |
| MTTP | 492 | CM000819.5 | 8 | 120820660 | 120871468 |
| PGAM2 | 316 | CM000829.5 | 18 | 48693832 | 48712787 |
| CAST | 1389 | CM000813.5 | 2 | 103255738 | 103378623 |
| LEPROT | 166 | CM000817.5 | 6 | 146974641 | 146987261 |
| ATP1B1 | 39 | CM000815.5 | 4 | 81912543 | 81937015 |
| APOE | 5 | CM000817.5 | 6 | 51373113 | 51375333 |
| CTSK | 44 | CM000815.5 | 4 | 98389459 | 98404969 |
| FABP4 | 3 | CM000815.5 | 4 | 55096400 | 55101209 |
| GNRHR | 26 | CM000819.5 | 8 | 65470206 | 65488900 |
| IGF-1 | 563 | CM000816.5 | 5 | 81762027 | 81909253 |
| LIF | 58 | CM000825.5 | 14 | 47221540 | 47239513 |
| MTHFR | 262 | CM000817.5 | 6 | 71863637 | 71882118 |
| PPARGC1A | 6423 | CM000819.5 | 8 | 17841844 | 18527953 |
| PPARD | 328 | CM000818.5 | 7 | 31222533 | 31295221 |
| RETN | 5 | CM000813.5 | 2 | 71484164 | 71485577 |
| TNNI1 | 98 | CM000821.5 | 10 | 23788279 | 23800554 |
| TNNI2 | 17 | CM000813.5 | 2 | 1251566 | 1253883 |
| VDBP | 13 | CM000819.5 | 8 | 68326287 | 68364937 |
| PPYR1 | 51 | CM000825.5 | 14 | 88276340 | 88283720 |

## RESULTS AND DISCUSSION

More than 30,000 variants were identified by comparing the gene set of each breed to the reference genome assembly. Of these ~22,000 were SNPs, ~3,000 short insertions and ~1400 short deletions. Transitions/transversions ratio was 2,650 while missense/silent ratio resulting in 0.526. Furthermore, over 40% of intronic variants and ~45% of noncoding transcript variants were also identified (Table 1).

Among all variants detected in this study, more than 3,000 were shared among NS, LW and LAN while ~7,000 were unique for NS, ~2,000 for LW and ~6000 for LAN, showing a high degree of genetic variability among studied breeds. Among the 5,659 unshared Nero Siciliano SNPs, 856 were in homozygosity while 4803 were found in heterozygosity. Interestingly, 802 SNPs were novel while 4857 were previously submitted to the dbSNP (Fig. 1 and Table 2). Among these only five were classified as "high impact" mutations at the following genetic loci *PLIN1, TBC1D1, CTSB, PGAM2* and *PPYR1* (Table 3).

The *PLIN1* gene encodes a protein belonging to a family of structural proteins with regulatory functions on the deposition and mobilization of intracellular lipids. Mutations of these genes have been associated with fatness in mouse[10] and human[11] but few other phenotypes have been associated with this mutation so far, as evidenced by some papers by D'Avoli *et al.*, 2011[12] and Gol *et al.* 2015[13].

Domain Family member 1 (*TBC1D1*) is correlated with lipid mobilization in pigs[14]. We identified a missense mutation (rs339837205) in position 29485163 affecting the start codon (ATG → GTG, position 1331 by mRNA), which result in an amino acid change at the first position M [Met] ⇒ V [Val].

Porcine cathepsin B *(CTSB)* is correlated with growth, carcass and meat quality traits in heavy pigs[15,16]. We identified a insertion in position 15034647, but its contribution to the phenotypic variation remains to be elucidated.

Phosphoglycerate mutase 2 *(PGAM2)* is correlated with meat color, carcass and meat quality traits in heavy pigs[17]. A mutation at the *PGAM2* locus was identified, exclusively in LAN breed, at position 48709077 (g.48709077 T>C). This mutation, classified as splicing, was already deposited in dbSNP (release 150) with ID: rs330054959.

Pancreatic Polypeptide Receptor 1 *(PPYR1),* influence gastric and pancreatic secretion[18]. A mutation in alternative homozygosity at the *PPYR1* locus was identified exclusively in LAN (rs335709223) at position 88276518 but its contribution to the phenotypic variation remains to be elucidated.

None of the homozygous variants were predicted to have high-impact effects on the biological function of the corresponding protein, with the exception of a missense mutation g. 160773437 G>A (rs81219178) found at the MC4R locus (ENSSSCG00000004904), which determines an amino acid substitution at position 298 (Asp→Asn) of the protein.

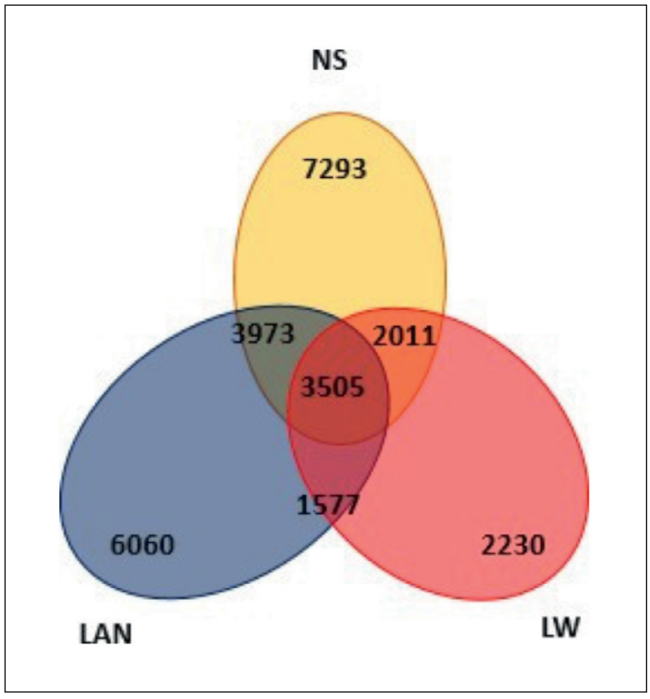


**Figure 1** - Venn-Diagram showing shared (internal) and unshared (external) variant (SNPs and short INDEL) detected in this analysis.

## CONCLUSION

In recent decades, the FAO (Food and Agriculture Organization) has expressed concern about the progressive replacement of local breeds with improved cosmopolitan breeds, since the latter cannot compete with the autochthonous ones characterized by rusticity, resistance to disease and adaptation to reduced food availability[19]. Many indigenous breeds show unique characteristics that can contribute to tackling

**Table 2** - SNPs identified in NS breed but not in LAN, LW and DU (Scrofa 11.1 Assembly).

| Category | Count | |
|---|---|---|
| | Homozygous | Heterozygous |
| Variants processed | 856 | 4803 |
| Novel variants | 113 (13.2) | 689 (14.3) |
| Existing variants | 743 (86.8) | 4114 (85.7) |

**Table 3** - Variants detected with "Hight impact" identified by SnpEff in NS pig.

| Chr | Position | Gene | Type | Effect/Impact | Ref | Alt |
|---|---|---|---|---|---|---|
| CM000818.5 | 55237669 | PLIN1 | INDEL | TCCCCCC\|frameshift_variant\|HIGH\|PLIN1\|transcript\| | TCCCCC | TCCCCCC |
| CM000819.5 | 29485163 | TBC1D1 | SNP | G\|start_lost\|HIGH\|TBC1D1\|transcript\| | A | G |
| CM000825.5 | 15034647 | CTSB | INDEL | CGGGGGGGG\|frameshift_variant\|HIGH\|CTSB\|transcript\| | CGGGGGGG | CGGGGGGGG |
| CM000825.5 | 88276518 | PPYR1 | SNP | C\|splice_acceptor_variant&intron_variant\|HIGH\|PPYR1\| | T | C |
| CM000829.5 | 48709077 | PGAM2 | SNP | C\|splice_donor_variant&intron_variant\|HIGH\|PGAM2\| | T | C |

the challenges linked to climate change, the growing demand for food connected to the increase in the world population and food security. In this study, we identified 7,293 unique variants in a subset of fatness-related genes in Nero Siciliano pig. Unshared SNPs and fixed mutations in this important breed could be the starting point for developing rapid and inexpensive molecular assays based on specific detection of DNA markers useful for breed differentiation as well as the authentication of the NS pig meat and commercial by-products. Methods for the authentication of breed-specific products are key tools to defend the added economic value of these products that represent the strategy to obtain a sustainable conservation of local animal genetic resources.

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Not applicable.

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