STUDY OF GENES INVOLVED IN THE GLYCOLYSIS OF THE SKELETAL MUSCLE TO IDENTIFY GENETIC MARKERS FOR MEAT QUALITY TRAITS IN PIGS

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Background

Improving meat quality is a major aim of the Italian pig breeding industry that is mainly focused on the production of dry-cured hams like Parma and San Daniele ham (Russo and Nanni Costa, 1995).
Glycolytic potential (GP) has been described as a biochemical measure useful to predict the quality of pig meat and its suitability for the dry-curing processing (Monin *et al.*, 1987; Nanni Costa *et al.* 2000a; Nanni Costa *et al.* 2000b). GP is defined as the quantity of glucide compounds of the muscle susceptible to conversion into lactic acid during the *post mortem* phase and is calculated according to the following formula: GP = 2([glycogen] + [glucose-6-phospate] + [glucose]) + [lactic acid] (Monin and Sellier, 1985). This parameter strongly influences ultimate pH (pH_u), which, in turn, affects other quality traits such as water holding capacity and meat colour. A very high level of GP is found in Hampshire pigs carrying the dominant *RN*$^-$ allele at the *RN* (Rendement Napole) locus whose effect is to increase the glycogen content of skeletal muscles *in vivo*. Fresh meat from these animals has lower pH_u, lower holding capacity and lower processing yield, especially in cooked hams (Sellier, 1998). Genome scanning and positional cloning resulted in the identification of the causative mutation of the *RN*$^-$ allele in the *PRKAG3* gene (Milan *et al.*, 2000). Moreover, other alleles of the *PRKAG3* gene have been shown to influence glycogen content in pigs (Ciobanu *et al.*, 2001). However, Malek *et al.* (2001) indicated the presence of other QTLs for GP, glycogen content, pH and water holding capacity in pigs and quantitative genetic studies showed a moderate heritability of these technological measures in different pig breeds (Sellier, 1998) and of GP in Large White (Larzul *et al.*, 1998). Thus, it is supposed that several other genes with small-medium effect may be involved in the determination of GP and of the technological parameters of the meat that are correlated with this biochemical measure.
A possible strategy to identify DNA markers associated to traits of economic importance is the candidate gene approach. A high level of GP may be caused by alterations of the glucose metabolism in skeletal muscle. Thus, genes coding for enzymes involved in the glycolysis of this tissue can be considered candidates for GP. Among these enzymes, glucose-6-phosphate isomerase (GPI) and muscle pyruvate kinase (PKM2) catalyse the second step and the last step of the Embden-Meyerhof glycolytic pathway, respectively. *GPI* is highly expressed in the skeletal muscle tissue and maps on porcine chromosome 6 close to the *RYR1* locus (Chowdary *et al.*, 1989). *PKM2* is an isoform specific for this tissue and has been physically mapped on porcine chromosome 7 (Davoli *et al.*, 2002).

Objectives

A research project was carried out to identify DNA markers associated with GP and other meat quality traits. For this aim we investigated two candidate genes, *PKM2* and *GPI*.

Methods

To amplify a fragment of the 3'-untranslated region of the porcine *PKM2* locus, PCR primers (forward: 5'-AGGCGGCTGCAGTAGTCG-3'; reverse: 5'-CCCCTTAGCCTCCCTCACTC-3') were designed on the sequence of a partial cDNA of this gene that was isolated from a porcine skeletal muscle cDNA library (Davoli *et al.*, 2002). PCR amplifications were carried out on a PT100 (MJ Research Inc., Watertown, MA, USA) thermal cycler in a final volume of 20 µl including 100 ng of porcine genomic DNA, 1 X PCR buffer, 1.5 mM $MgCl_2$, 10 pmol of each primer, 250 µM of each dNTP and 1 U of *Taq* DNA polymerase (Roche Diagnostics, Mannheim, Germany). The cycling profile was the following: first denaturation step at 95 °C for 5 min; 35 cycles of 30 s at 95 °C, 30 s at 58 °C and 30 s at 72 °C; final extension at 72 °C for 5 min. SSCP analysis was performed according to the protocol described by Fontanesi *et al.* (2001). Mendelian inheritance of the polymorphism was confirmed in three-generation pig families. For *GPI*, we analysed an amplified product length polymorphism as already described by Jiang and Gibson (1998). PCR products were electrophoresed on 10% polyacrylamide gels. Animals were 507 commercial pigs (Duroc x [Landrace x Large White]) subjected to an experiment with transport condition controlled (Nanni Costa *et al.*, 1999), coming from one farm and slaughtered at about 165 kg live weight in the same abattoir. At 1 hr *post mortem*, pH_1 with Crison pH-meter equipped with an Ingold Xerolite electrode, and internal reflectance with a Fiber Optic Probe (FOP_1; TBL, Leeds, UK) on *biceps femoris* muscles were measured. Colour was assessed on an exposed surface of this muscle by CIELAB co-ordinates with a Minolta Chromameter II (light source C, 8 mm diameter). At 24 hr *post mortem*, pH_u, FOP_u and colour measurements were taken on the same muscle. Moreover, on a sample of *longissimus thoracis* muscle collected at the level of the last rib drip loss and cooking losses were assessed using the method of Honikel (1987). A sample of *biceps femoris* muscle was collected at 1 hr and at 24 hrs after death, frozen in liquid nitrogen and later freeze-dried. Glycogen, glucose, glucose-6-phosphate and lactic acid content were separately determined in the sample (Bergmeyer, 1974). GP at 1 hr and at 24 hrs *post mortem* were calculated according to Monin and Sellier (1985) and expressed as µmol of lactic acid equivalent per g of fresh muscle, assuming moisture content of 75% (Talmant *et al*, 1989). Among the 507 pigs, 2-4 animals for each class/treatment, described by Nanni Costa *et al.* (1999), were selected for a total of 60 pigs. Total genomic DNA was extracted from freeze-dried muscle samples of these pigs (Sambrook *et al.*, 1989) and analysed for the polymorphisms at the *PKM2* and *GPI* loci. Furthermore, these animals were typed to exclude the presence of the *n* allele of the *RYR1* gene (Fujii *et al.*, 1991; Russo *et al.*, 1993) and of the *RN*$^-$ allele of the *RN* locus (*PRKAG3* gene; Milan *et al.*, 2000). Association between genotype at the *PKM2* and *GPI* loci and meat quality technological parameters were evaluated by means of the GLM procedure of SAS (1994). The model included the fixed effect of the genotype of the *PKM2* or *GPI* locus, pre-slaughter condition (loading method, stocking density, truck deck) and sex and the random effect of day of slaughtering.

Results and discussion

Amplifying porcine genomic DNA for the *GPI* locus, we confirmed the presence of a biallelic polymorphism (allele *1* and allele *2*) that, as described by Jang and Gibson (1998), is due to a 20 bp duplication in the sixth intron of the gene. Moreover we identified a novel SSCP with two alleles (allele *1* and allele *2*) in a fragment of 128 bp of the 3'-untranslated region of the *PKM2* gene. These markers were typed in a subset of animals (No. 60) that were part of a transportation experiment aimed to investigate effects of pre-slaughter handling on glycogen and lactate contents, and other related meat quality traits (Nanni Costa *et al.*, 1999). These animals did not carry the negative alleles of the two major loci, namely *RYR1* and *RN* that influence meat quality traits. The effects of *PKM2* and *GPI* genotypes on GP and meat quality traits are shown in Table 1. At 1 hr *post mortem*, genotype *2/2* of *PKM2* had significantly higher GP and glycogen content than genotypes *1/1* and *1/2*, which did not differ in that traits. Genotype *2/2* had also the lowest lactate content compared to the other two genotypes but did

not reach the statistical significance. According to the glycogen and lactate contents, meat from pigs with genotype *2/2* at the *PKM2* locus showed a higher pH_1 value and lower L^*_1, a^*_1 and FOP_1 values. Nevertheless, differences among genotypes were not significant. There were not significant differences among the *PKM2* genotypes in meat quality traits measured at 24 hrs *post mortem* even if genotype *2/2* showed, again, the highest value of GP for which the lactate instead of the glycogen content, as expected, contributed to this result. In general, the consequences of meat quality due to the highest GP at 1 hr *post mortem* in pigs with *PKM2 2/2* genotype were not relevant because the level of this parameter was not sufficient to lead to a low pH_u. *GPI* genotypes did not influence significantly GP and meat quality traits with the only exception of lactate content at 1 hr *post mortem*. Genotype *2/2* at this locus had the highest value, genotype *1/1* the lowest, and genotype *1/2* showed an intermediate value compared to the homozygote pigs. According to this data, genotype *2/2* showed the lowest value for pH_1 and the highest for FOP_1. At 24 hrs *post mortem* differences among *GPI* genotypes in meat quality traits were very small.

Conclusions

In general, these preliminary results may indicate that variations of GP due to different genotypes for *PKM2* and *GPI* enzymes seem to affect meat quality measurements after 1 hr *post mortem* with few consequences for the ultimate meat quality characteristics. Further experiments are needed to evaluate these DNA markers as predictors of meat quality traits. Moreover, investigations on these two polymorphisms should be carried out considering also other economically important traits. As a matter of fact, *GPI* has been associated also with growth traits (Clamp *et al.*, 1992) while *PKM2* is localized on chromosome 7 in which several studies have demonstrated the presence of important QTLs for back-fat thickness, intramuscular fat, disease resistance and growth traits.

Pertinent literature

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Table 1. Effect of the *PKM2* and *GPI* genotypes on glycolytic potential and meat quality traits (least squares means ± S.E.)

Loci	*PKM2*			*GPI*°		
Genotypes	*1/1*	*1/2*	*2/2*	*1/1*	*1/2*	*2/2*
No. of pigs	11	30	19	20	24	13
1 hr *post mortem*						
Glycolytic potential§	152.75 ± 8.35[a]	156.75 ± 6.41[a]	176.33 ± 7.40[b]	162.32 ± 6.84	157.56 ± 7.19	166.99 ± 8.61
Glycogen§	51.48 ± 4.60[a]	58.52 ± 3.53[a]	67.76 ± 4.08[b]	62.34 ± 3.79	56.78 ± 3.99	57.54 ± 4.78
Lactate§	38.66 ± 4.91	31.59 ± 3.77	31.01 ± 4.36	28.43 ± 3.54[a]	34.74 ± 3.73[ab]	41.75 ± 4.46[b]
pH_1	6.37 ± 0.06	6.42 ± 0.05	6.50 ± 0.05	6.48 ± 0.05	6.41 ± 0.05	6.37 ± 0.06
L^*_1	39.17 ± 0.82	38.47 ± 0.63	37.59 ± 0.73	38.76 ± 0.64	38.26 ± 0.67	37.75 ± 0.80
a^*_1	6.52 ± 0.66	6.43 ± 0.51	6.39 ± 0.59	6.64 ± 0.50	6.28 ± 0.53	6.27 ± 0.63
FOP_1	22.15 ± 1.38	20.75 ± 1.06	19.76 ± 1.22	20.39 ± 1.02	20.19 ± 1.08	22.86 ± 1.29
24 hrs *post mortem*						
Glycolytic potential§	129.27 ± 5.72	133.07 ± 4.39	139.76 ± 5.07	130.36 ± 4.39	136.45 ± 4.62	138.58 ± 5.53
Glycogen§	8.46 ± 0.87	11.72 ± 0.89	11.38 ± 0.85	11.15 ± 0.92	10.34 ± 0.89	10.60 ± 0.91
Lactate§	97.66 ± 4.55	95.87 ± 3.49	102.55 ± 4.04	94.94 ± 3.44	99.81 ± 3.62	103.67 ± 4.33
pH_u	5.51 ± 0.02	5.50 ± 0.02	5.47 ± 0.02	5.49 ± 0.02	5.51 ± 0.02	5.49 ± 0.02
L^*_u	51.24 ± 1.33	49.61 ± 1.02	49.76 ± 1.18	49.25 ± 1.00	51.24 ± 1.05	50.00 ± 1.26
a^*_u	10.23 ± 0.65	9.67 ± 0.50	9.03 ± 0.58	9.66 ± 0.50	9.27 ± 0.53	10.13 ± 0.63
FOP_u	34.91 ± 1.53	36.57 ± 1.17	35.49 ± 1.35	36.84 ± 1.15	34.55 ± 1.20	35.62 ± 1.44
Drip loss (%)	3.67 ± 0.45	3.94 ± 0.35	3.53 ± 0.40	3.57 ± 0.34	3.90 ± 0.36	3.88 ± 0.43
Cooking loss (%)	24.19 ± 0.82	24.35 ± 0.63	23.99 ± 0.73	24.32 ± 0.63	23.88 ± 0.66	24.44 ± 0.79

a,b: $P<0.05$.°: typed only 57 pigs. §: expressed in µmol/g.