

ANALYSIS OF *PRKAG3* GENE POLYMORPHISMS IN ITALIAN AUTOCHTHONOUS PIG BREEDS

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ABSTRACT

The *PRKAG3* gene encodes for the regulatory gamma 3 subunit of adenosine monophosphate-activated protein kinase (AMPK) protein that acts as a regulator of carbohydrate and fat metabolism. Several single nucleotide polymorphisms (SNPs) have been identified in the porcine *PRKAG3* gene. Two of them (c.595A>G or p.I199V and c.599G>A or p.R200Q) have been associated with variability of meat quality and carcass traits. In this study, we investigated the frequency of the c.595A>G and c.599G>A SNPs in 372 animals of five Italian autochthonous pig breeds (Apulo-Calabrese, Casertana, Cinta Senese, Mora Romagnola and Nero Siciliano). Genomic DNA was extracted from hair roots or blood and SNPs genotyping was performed by PCR-RFLP protocols. The c.599A (p.200Q) allele was carried by only 3 Nero Siciliano pigs. All five breeds were polymorphic at the c.595A>G site. The c.595A (p.I199) allele was less frequent in the analysed breeds with minor allele frequency ranging from 0.144 (Nero Siciliano) to 0.464 (Casertana). Based on identified allele frequencies, the c.595A>G SNP can be useful in association studies with meat, carcass and ham quality traits in the Italian local pig breeds.

Key words: *PRKAG3* gene / pigs / breeds / allele / genotype frequencies

1 INTRODUCTION

The *PRKAG3* gene encodes for the regulatory gamma 3 subunit of adenosine monophosphate-activated protein kinase (AMPK) that plays key roles as regulator of carbohydrate and fat metabolism in mammalian skeletal muscle cells (Barnes and Zierath, 2005). Several polymorphisms have been identified in the porcine *PRKAG3* gene (Milan *et al.*, 2000; Ciobanu *et al.*, 2001, Ryan *et al.*, 2012). Of these mutations, the c.595A>G and c.599G>A single nucleotide polymorphisms (SNPs) (causing the p.I199V and p.R200Q aminoacid substitutions, respectively) were the most studied in different pig breeds and populations. The c.595A>G SNP has been associated with variation of different traits including water holding capacity, pH, colour, carcass traits and composition, and several quality traits of dry-cured ham

(Ciobanu *et al.*, 2001, Gou *et al.*, 2012; Santé-Lhoutellier *et al.*, 2012; Škrlep *et al.*, 2012). The pigs that carry the c.599A (p.200Q) allele have higher skeletal muscle glycogen content and lower Rendement Napole technological yield (this allele was previously referred to be the *RN* allele) compared to animals that do not carry this allele. The c.599A allele is responsible of the so-called acid meat defect, characterised by very low ultimate pH, and/or Hampshire type meat, due to the common presence of the p.200Q allele in Hampshire and Hampshire derived lines (Monin and Sellier, 1985; Le Roy *et al.*, 1990; Milan *et al.*, 2000; Ciobanu *et al.*, 2001; Lundström *et al.*, 2006; Cherel *et al.*, 2010; Scheffler *et al.*, 2011).

The aim of the current study was to investigate the allele and genotype frequencies of the c.595A>G and c.599G>A *PRKAG3* SNPs in five Italian local pig breeds.

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2 MATERIALS AND METHODS

2.1 ANIMALS, GENOMIC DNA EXTRACTION AND GENOTYPING

Samples were collected from Apulo-Calabrese (n = 70), Casertana (n = 28), Cinta Senese (n = 75), Mora Romagnola (n = 91) and Nero Siciliano (n = 108) pigs. Sampled animals were from different herds and registered to the Anagraphic Book of the National Pig Breeders Association (ANAS; <http://www.anas.it>). Genomic DNA was extracted from hair roots or blood and SNP genotyping was performed by PCR-RFLP analyses (Ciobanu *et al.*, 2001; Fontanesi *et al.*, 2003). Briefly, the PCR product of 259 bp (Ciobanu *et al.*, 2001) was digested with *MbiI* (c.599G>A) and *BsaHI* (c.595A>G). PCR-RFLP products were resolved on 2.5% agarose gels visualized with 1xGelRed Nucleic Acid Gel Stain.

2.2 STATISTICAL ANALYSIS

Allele and genotype frequencies of the two analysed polymorphisms were determined. Hardy-Weinberg equilibrium was evaluated using the HWE software program (Linkage Utility Programs, Rockefeller University, New York, NY, USA).

3 RESULTS AND DISCUSSION

The region of origin and demographic data (year 2011) of the analysed five breeds is reported in Table 1 (ANAS, 2012). These local breeds belong to the Mediterranean type (southern European origin), characterized by a coloured coat and a high tendency for increased intramuscular fat, or are intermediate between European and Indochinese pigs (Franci and Pugliese, 2007; Megens *et al.*, 2008). Among the investigated breeds, the Nero Siciliano population may have been crossbred heavily in recent times with commercial western European

breeds (Megens *et al.*, 2008; Fontanesi *et al.*, 2010). All investigated local breeds are adapted to local climate and marginal conditions and they are less efficient for productive (growth rate and feed conversion) and reproductive performances (number of piglets born alive and teat numbers) and carcass traits (tendency to fatness) than other improved cosmopolitan breeds. However, the local breeds have in general better meat quality traits such as more red colour and lower cooking loss than the improved ones (Franci and Pugliese, 2007).

We analysed the two SNPs of the *PRKAG3* gene located in a highly conserved region of the encoded protein which acts as a sensor of cellular energy status (Milan *et al.*, 2000). Pigs from Apulo-Calabrese, Casertana, Cinta Senese and Mora Romagnola were homozygous for the wild type c.599G (p.200R) allele. The c.599A (p.200Q) allele was carried, in heterozygous state (c.599AG genotype) only by 3 Nero Siciliano pigs. This data suggested that Hampshire or Hampshire-derived lines could have contributed to shaping Nero Siciliano genetic pool, as in part supported by other studies on coat colour (Fontanesi *et al.*, 2010). Due to the low frequency of the c.599A allele in the Nero Siciliano (equal to 0.014), this polymorphic site might be of limited importance in determining the variability of meat and carcass quality traits in this breed. However, this breed should be further monitored to avoid excessive diffusion of this negative allele in the population.

All breeds were polymorphic at the c.595A>G (p.I199V) polymorphism (Table 2). This SNP does not deviate from Hardy-Weinberg equilibrium in the genotyped populations ($P > 0.05$). The c.595A (p.I199) allele was less frequent in all analysed breeds, in agreement with earlier studies performed in other commercial breeds and populations reared in Italy (Fontanesi *et al.*, 2003; 2008) and in the majority of modern breeds and crosses. In the Italian local breeds the frequency of the minor allele (c.595A), that has been shown to be favourable for some meat quality traits such as pH and water holding capacity (Ciobanu *et al.*, 2001; Fontanesi *et al.*, 2008), ranged from 0.144 (Nero Siciliano) to 0.464 (Ca-

Table 1: Details of Italian local breeds registered to the Anagraphic Book of the National Pig Breeders Association. The population data were referred to December 31, 2011 (ANAS, 2012)

Local pig breeds	Italian region of origin	Coat colour of registered pigs	Number of boars	Number of sows	Number of young candidates
Apulo-Calabrese	Calabria	Black	93	527	3,523
Casertana	Campania	Dark gray	33	80	323
Cinta Senese	Tuscany	Black with white belt	170	1,035	1,552
Mora Romagnola	Romagna	Black	67	235	683
Nero Siciliano	Sicily	Black	45	523	2,460

Table 2: Minor allele (MAF) and genotype frequencies of the c.595A>G SNP in five Italian local pig breeds

Local pig breeds	Number of pigs	c.595A>G (p.I199V) SNP		Genotype frequency		
		MAF (allele)		c.595AA	c.595AG	c.595GG
Apulo-Calabrese	70	0.350 (c.595A)		0.171	0.357	0.472
Casertana	28	0.464 (c.595A)		0.286	0.357	0.357
Cinta Senese	75	0.350 (c.595A)		0.083	0.533	0.383
Mora Romagnola	91	0.154 (c.595A)		0.011	0.286	0.703
Nero Siciliano	108	0.144 (c.595A)		0.028	0.231	0.741
Total	372					

sertana). Combining the two investigated polymorphic sites, we identified 3 haplotypes, namely [199V:200R], [199I:200R] and, in Nero Siciliano, [199V:200Q].

4 CONCLUSIONS

The results indicated the absence of the negative c.599A allele in Italian local pig breeds, except for Nero Siciliano. Based on identified allele frequencies, the c.595A>G SNP can be useful in association studies with production, meat, carcass and ham quality traits in the Italian local pig breeds.

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