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A further look on genetic basis of carcass fat deposition in pigs of 'Casertana' ancient autochthonous genetic type

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Abstract. 'Casertana' pig ancient autochthonous genetic type (AAGT) has been considered in the years within wide programme of safeguard and rescue promoted by ConSDABI. One of the most relevant aims of this program is to search for gene variation related to quantity and technological, nutritive and organoleptic quality of fat in order to satisfy consumer's request. In the present work, 100 AAGT Casertana pigs were genotyped for 8 SNP at *loci* involved in the regulation of adipose tissue deposition [DECR1, FASN, MC4R, SCD and H-FABP haplotype (H-FABP1, H-FABP2, H-FABP3)] in order to carry out an association study. with some fatness characteristics of carcass. Statistical analysis was performed using the GLM procedure of the SAS package. The results showed association between HFABP haplotype and the majority of considered phenotypic traits. In detail, the effect of this haplotype was significant on the total weight of separable fat in carcass (P=0.002), total weight of adipose cuts (P=0.006) and back fat thickness measured at level of 1st thoracic vertebra (Th1) (P=0.056), 15th thoracic (Th15) (P= 0.020) and between the 6th lumbar vertebra and sacrum (L6 – S) (P=0.007). FASN showed an effect on belly and jowl fat (P≤0.05). For DECR1 CC genotype influenced the weight of belly cut. Further investigations are ongoing for an operative utilisation of the H-FABP haplotype, FASN and DECR1 genes as molecular markers (candidates) in proper molecular assisted selection (MAS) plans.

Keywords. Casertana 'AAGT' – Fat traits – SNPs – Haplotype.

Une perspective sur les bases génétiques du dépôt de graisse dans la carcasse des porcs du type génétique autochtone ancien 'Casertana'

Résumé. *Le porc 'Casertana', type génétique autochtone ancien (TGAA) du "bioterritorio" de la Campanie, est depuis des années l'objet d'un programme de protection et de valorisation mis en œuvre par le ConSDABI SUB NFP.I - FAO. Parmi les objectifs de ce programme il y a celui d'identifier des variantes des gènes associés à la quantité et à la qualité technologique, nutritionnelle et organoleptique des matières grasses pour répondre aux besoins du consommateur. Dans cet article, nous avons étudié 8 SNP dans les loci candidats au déterminisme quantitatif du tissu adipeux [DECR1, FASN, MC4R, SCD et haplotype H-FABP (H-FABP1, H-FABP2, H-FABP3)]. 100 porcs TGAA 'Casertana' pour lesquels certains relevés étaient disponibles concernant des sections, ont été génotypés afin d'effectuer une étude d'association avec quelques caractéristiques de l'adiposité. L'analyse statistique a été réalisée en utilisant la procédure GML du SAS. Les résultats suggèrent une association entre l'haplotype H-FABP et la plupart des caractères en question. En particulier, l'effet de l'haplotype H-FABP est significatif sur le poids total des morceaux de gras (P=0,006), sur les gras séparables (P =0,002) et sur l'épaisseur de gras mesurée à la 1ère vertèbre thoracique (Th1) (P=0,056), à la 15ème vertèbre thoracique (Th15) (P= 0,020) et entre la 6ème vertèbre lombaire et le sacrum (L6 – S) (P=0,007). En outre, FASN a des associations avec le lard de la poitrine et le lard de la bajoue (P ≤ 0,05). D'autres recherches sont en cours pour une utilisation opérationnelle des haplotypes H-FABP et des gènes FASN et DECR1 comme marqueurs prometteurs ('candidats') dans les plans de sélection assistée par marqueurs moléculaires (MAS).*

Mots-clés. *TGAA 'Casertana' – Caractéristique de l'adiposité – SNP – Haplotype.*

I – Introduction

'Casertana' (CT) ancient autochthonous genetic type (AAGT) is a black pig of ancient origins. It is object of considerable interest for its many peculiarities, like: good aptitude to grazing with ability to utilize poor feed; appreciable organoleptic and healthy quality of meat which is particularly suitable to obtain valuable local products (Matassino *et al*., 1968; Colatruglio *et al*., 1994; Girolami *et al.,* 1996; Matassino *et al*., 2006; Barone *et al*., 2008). In the last years, the awareness of the nutritional issue has increased: healthy quality is the element that more concerns the consumer. In this context, the study of quanti-qualitative characteristics of the adipose tissue as well as of the factors influencing them in farm animals, especially of pig, has become increasingly important. Furthermore ongoing research at ConSDABI SUB NFP.I - FAO on pig AAGTs is corroborating the hypothesis to consider pig as a valid model to study human obesity. These pigs, characterized by a thicker back fat than cosmopolite breeds, can constitute an interesting resource to contribute to the knowledge of genetic factors involved in obesity; moreover, AAGTs can constitute a genetic reserve suitable to rescue organoleptic properties penalized in cosmopolite breeds. Different approaches are used to identify molecular markers linked to traits associated to adipose tissue deposition in pig: (i) candidate gene; (ii) QTL identification; (iii) combination of (i) and (ii); (iv) transcriptome analysis (Davoli *et al*., 2009). Various genes involved in quali-quantitative characteristics of muscle and adipose tissue have been identified. The aim of the present contribute was to evaluate, in CT pig, possible associations between 8 SNPs at *loci* involved in regulation of adipose tissue deposition [DECR1, FASN, MC4R, SCD and H-FABP haplotype (H-FABP1, H-FABP2, H-FABP3)] and some fatness characteristics of carcass; these *loci* are known in literature for their significant association with some fatness traits (Gerbens *et al*., 1997; Kim *et al*., 2000; Wimmers *et al.,* 2002; Munoz *et al*., 2003; Amills *et al*., 2005; Matassino *et al*., 2007 and 2009).

II – Materials and methods

DNA was extracted from blood and muscle samples of 100 subjects of CT AAGT, reared at experimental Farm of ConSDABI SUB NFP.I - FAO. Genotyping for SNPs considered at DECR1, FASN, H-FABP, MC4R and SCD *loci* was carried out by PCR-RFLP method according to literature protocols (Table 1).

Table 1. SNPs investigated in 'candidate' *loci* object of study

SNP	*LOCUS*				BIBLIOGRAPHIC REFERENCE
	ACRONYM	DENOMINATION	FUNCTION	CHROMOSOME	
C(163)G	DECR1	2,4 - Dienoyl reductase 1 mithocondrial	Encodes for an enzyme involved in unsaturated fatty acid beta-oxidation	4	Davoli *et al*., 2002
T265C	FASN	Fatty acid synthase	Enzyme key in the conversion of acetyl-CoA and malonyl-coA into long-chain satured fatty acids	12	Munoz *et al*., 2003
C(1811)G H-FABP1 (D/d) T(1324)C H-FABP2 (H/h) T(1970)C H-FABP3 (A/a)	H-FABP	Heart fatty acid - binding protein	Trafficking of fatty acids at level of cardiac and striate muscle as well as lactating mammary gland	6	Gerbens *et al*., 1997
G(298)A	MC4R	Melanocortin 4 receptor	Control of uptake capacity and growth rate	1	Kim *et al*., 2000
T(233)C SCD1 C(641)T SCD3	SCD	Stearoyl CoA Desaturase	Encodes for an enzyme key in the monounsaturated fatty acid biosynthesis	14	Ren *et al.*, 2004

For each SNP the allelic and genotypic frequencies were estimated. Concerning H-FABP, 12 out of 27 possible haplotypes were individuated, only 6 of which (HHddAa, HHddAA, HHDdAa, HHDdAA, HHddaa, HhDdAa) were used for statistical elaboration because of the insufficient numerousness of the others; for each fatness indicator 15 possible pair-wise comparisons were carried out. In the present contribute the following parameters at carcass dissection were considered as adiposity indicators: (i) total weight of adipose cuts (with and without 'jowl'); (ii) total weight of separable fat; (iii) thickness (mm) of back fat measured at level of: 1st thoracic vertebra (Th1), 15th thoracic vertebra (Th15) and between the 6th lumbar vertebra and sacrum (L6 - S).

Statistical elaboration, concerning the association between genotypic and phenotypic data, was performed with the following factorial model of covariance analysis with fixed factors utilizing SAS 9.1v software:

$$Y_{ijK} = \mu + b_1x_1 + b_2x_2 + b_3x_3 + \alpha_i + sex_j + e_{ijK}$$

where:

μ = constant common to all observations (overall mean);

x_1 = weight of refrigerated carcass after 72 hours of refrigeration (covariate);

x_2 = date of slaughtering (covariate);

x_3 = age of the pig at slaughtering (covariate);

α_i = fixed effect common to all observation relative to ith genotype (i = 1,2,3)/haplotype (i=1, 2, 3, 4, 5, 6);

sex_j = fixed effect common to all observations related to jth sex (j = 1,2);

e_{ijK} = random error.

III – Results and discussion

From statistical elaboration it emerged a significant effect of some *loci* on carcass fatness. *H-FABP*. The comparison among the considered haplotypes highlighted that the pig with HHDdAA or HHDdAa haplotype, when compared with a subject with HHddAA or HHddAa or HHddaa haplotype, gives a higher adiposity estimated through any detected parameters (Table 2). In particular, the difference of: (i) back fat thickness can vary from about 9 (HHDdAa *vs* HHddAa; P=0.020) to about 17 mm (HHDdAA *vs* HHddAA; P=0.002); (ii) total fat cuts (without belly) can range from 6.5 (HHDdAA *vs* HHddAa; P=0.075) to about 16 kg (HHDdAa *vs* HHddaa; P= 0.002); (iii) of separable cuts can vary from about 6.0 (HHDdAA *vs* HHddAa; P=0.073) to 13.5 kg (HHDdAa *vs* HHddAA; P=0.0001).

Table 2. Some parameters of fatness: significant comparisons between 'H-FABP' haplotypes

Haplotype		Parameter											
		Backfat thickness, mm						'Fat cuts', kg				'Total separable fat', kg	
Reference (I)	Comparison (J)	'Th1'		'Th15'		'L6-S'		'Total'		'Total without Jowl'			
		Δ (I-J)	P-value	Δ (I-J)	P-value	Δ (I-J)	P-value	Δ (I-J)	P-value	Δ (I-J)	P-value	Δ (I-J)	P-value
HHDdAA	HHddAA	12.443	0.031	15.113	0.004	16.949	0.002	8.047	0.028	8.197	0.042	10.542	0.005
	HHddAa	10.958	0.035	11.934	0.011	13.080	0.006	6.565	0.048	6.493	0.075	5.987	0.073
	HHddaa	15.412	0.043	15.277	0.027	16.563	0.017	5.154	0,287	13.485	0.013	6.677	0.173
HHDdAa	HHddAA	11.321	0.031	11.640	0.015	13.332	0.006	9.784	0.004	10.538	0.004	13.490	0.0001
	HHddAa	9.836	0.022	8.461	0.029	9.463	0.016	8.302	0.003	8.835	0.004	8.935	0.002
	HHddaa	14.290	0.043	11.804	0.063	12.946	0.043	6.890	0.126	15.827	0.002	9.625	0.036

The biological and operative importance to consider the effect of a haplotype on a quali-quantitative trait has been widely debated (Matassino *et al.*,1993; Zullo *et al.*, 1994). Indeed, according to these authors, the global genotype has a semantic value for marker assisted selection (MAS). Within H-FABP, it was believed convenient to individuate a possible effect of genotype at single *locus* on adiposity using single nucleotide polymorphism. The effect was significant only for D/d polymorphism: the subject with dd genotype gave a significant lower adiposity in comparison with heterozygote for the majority of the parameters considered, with a decrease equal to: (i) about 10 mm for back fat thickness (P= 0.003 at Th1 region; P= 0.001 at Th15 and L6-S regions); (ii) about 8 kg for total fat cuts and total separable fat (P<0.001).

FASN. The effect was significant on the weight of belly fat cut (P=0.051) and on that of separable fat from jowl (P=0.048). It is interesting to observe that the subject with TT genotype, in comparison with that with CC or CT genotype, gives a significant (P≤0.05): (i) lower weight of belly cut [the difference is equal to 0.895 kg and 1.072 kg for CC *vs* TT and CT *vs* TT comparisons, respectively]; (ii) higher weight of separable fat from jowl (difference equal to - 0.439 kg and - 0.454 for CC *vs* TT and CT *vs* TT comparisons, respectively).

DECR1. The effect of this gene was near to critical limit of significance (P= 0.072) on jowl; from the comparison between genotypes it emerges that the pig with CC genotype gives a lighter belly than the subject with CG or GG genotype; the comparison was significant (P= 0.022) for CC *vs* CG with a difference of -0.902 kg and tendentially significant (P=0.135) for CC *vs* GG with a difference of - 0.608 kg.

SCD. This gene tended to influence the weight of separable fat from coppa adipose cut (P= 0.148), back fat (P= 0.144) and total separable fat (P= 0.132).

MC4R. A tendency to significance (P= 0.179) was evidenced for separable fat from jowl.

IV –Conclusions

In the limits of the observation field, the results suggest that H-FABP haplotype significantly affects the parameters used for the estimation of carcass adiposity, determining differences among subjects with different haplotypes. The pig with HHddAA would be the less physiological obese for the total of adipose cuts without jowl and of separable fat. FASN locus would cause a differential fat deposition probably associated to the effect of TT genotype showing a variable lipogenetic aptitude in different anatomical regions of the body. For DECR1 locus, CC genotype would influence the weight of belly fat. Further investigations are ongoing for an operative utilisation of the H-FABP haplotype and FASN and DECR1 genes as molecular markers (candidates) in proper MAS plans.

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