Ann. Anim. Sci., Vol. 10, No. 1 (2010) 17–25

A SUBSET OF CANDIDATE POLYMORPHISMS IDENTIFIED BY 52 SNPs MINI-ARRAY IN TWO DUROC SUB-POPULATIONS REVEALED SIGNIFICANT DIFFERENCES IN SNP ALLELE DISTRIBUTIONS*

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Abstract

Although each breed is characterized by a set of established traits and qualities, in breeding strategies led by national programmes or commercial companies, animals belonging to the same breed may differ significantly because of different origin and breeding strategy. It is commonly known that Duroc fatteners imported to Poland from Danish breeding companies when compared to Duroc pigs bred in Poland differ significantly in meat quality parameters. In this report we try to determine whether the representatives of these two Duroc sub-populations differ in allele distributions in 52 SNPs, localized in 46 genes, chosen from literature as potentially influencing pork yield and quality. Using chi square test we have found that 27 SNPs have significantly different allele distributions. Among them 17 showed an adverse trend of allele frequency, which means that allele 1 was major in one Duroc population and minor in another Duroc population and this difference was significant at P<0.01. These loci can be considered as promising candidate genes involved in pork quality variation.

Key words: pig, Duroc breed, meat quality

Improving meat quality is one of the most challenging tasks in commercial pig breeding. Meat quality parameters are difficult and expensive to measure accurately on a large number of pigs in a progeny testing scheme. Using a molecular approach, several SNPs, namely, the missense mutation in *RYR1* (Fujii et al., 1992) and in

*This work was financed from UWM grant no. 0105-804.

PRKAG3 (Milan et al., 2000) have major effects on lean meat content and meat quality, as well as the point mutation in intron 3 of *IGF2* (Van Laere et al., 2003) underlying a major QTL for muscle growth and lean meat content.

Duroc is recognized as a model breed for meat quality traits and it is used to construct national or commercial breeding programmes. Although each breed is characterized by a set of established traits and qualities, animals of same breed may differ significantly, depending on breeding strategy and its advancement. We hypothesized that these differences should be expressed by SNP allele distributions. SNPs showing the most significant differences in allele frequencies could be promising candidate markers potentially useful in marker assisted selection. The aim of this study was to check whether allele frequency of 52 SNPs identified by mini-array differs significantly between two Duroc populations of different origin. According to Kamiński et al. (2008), these SNPs are believed to represent the most important genes potentially associated with pork yield and quality.

Material and methods

A total of 78 unrelated Duroc fatteners (38 Danish and 40 Polish) were included in the study. Blood samples were taken to isolate genomic DNA by MasterPure Genomic Purification Kit (Epicentre). All animals were genotyped for 52 SNPs by the SNiPORK mini-array (Table 1) based on Arrayed Primer Extension (APEX) technology (Kamiński et al., 2008).

Results

Using chi square test we found that 27 SNPs have significantly ($P<0.01$) different allele distributions. Among them 17 showed an adverse trend of allele frequency (marked in bold in Table 2), which means that allele 1 was major in one Duroc sub-population and minor in another Duroc sub-population and this difference was significant at $P<0.01$.

In four cases the differences were extreme: TNNT2 (skeletal muscle troponin, allele 1–0.07 vs 0.91), PPARG (peroxisome proliferator activated receptor gamma, allele 1–0.74 vs 0.01), TYR (tyrosinase, allele 1–0.79 vs 0.10) and PKM2 (pyruvate kinase 2 muscle, 0.78 vs 0.21). In another four loci SNPs turned to be monomorphic in one population and polymorphic in another: CYP21, CYP2E1 and DECR1. In the remaining loci the differences between allele frequencies were smaller, but also at statistically significant level ($P<0.01$), for example: CAST (calpastatin, allele 1–0.80 vs 0.29), H FABP (heart fatty acid binding protein – 0.24 vs 0.78) MYOP (myopalladin – 0.04 vs 0.56), MC4R (melanocortin 4 receptor – 0.38 vs 0.03). As fatteners were chosen randomly and were the offspring of many parent couples, the possible influence of parent's allele on allele distribution can be ignored.

Table 1. Molecular definition of 52 SNiPORK SNPs

Locus symbol	SSC	Locus name	GenBank number	SNP position	SNP significance
1	2	3	4	5	6
ACSL	X	acyl CoA synthase long chain 4	DQ144454	G2645A	3'UTR
ADP	-	adiponectin	AJ849536	G1719A\	V60I
CAST	2	calpastatin	DQ339697 + AY594692	A408G	N167S
CAST	2	calpastatin	DD217638	A47G	R339K
CAST	2	calpastatin	DD217639	A499C	R728S
CRH	4	corticotropin releasing hormone	AF440229	G400A	R28Q
CSTB	13	cystatin	AJ315561	A367G	D63N
CYP21	7	steroid 21 hydroxylase	M83939	A2991C	intron splicing site
CYP2E1	14	cytochrome p450 2E1	AJ697882	C2412T	5'-flanking
CYP2E1	14	cytochrome p450 2E1	AJ697882	G744A	A475T
DECR1	4	mitochondrial 2,4 dienoyl CoA reductase	AF335499	G90C	V54L
DES	15	desmin	AF136188	C749T	silent
ESR1	1	estrogen receptor alpha	AF034974	T472C	silent
ESR2	1	estrogen receptor beta	AY357117	G388A	M317V
FHL3	-	four and half LIM only protein	AY377857	A312G	G75R
GAA	12	alpha acid glucosidase	AJ557226	C38T	silent
GH	12	growth hormone	U58113	A306T	TATA box
GH	12	growth hormone	U58113	G200T	SP1 binding
GH	12	growth hormone	AY727040	A485	R22Q
GHR	16	growth hormone receptor	DQ388035	A155G	silent R51
GYS1	6	glycogen synthase	AJ507152	G418A	intron 14
H FABP	6	heart fatty acid binding protein	X98558, own sequencing	T1324del	5' flanking
H FABP	6	heart fatty acid binding protein	Y16180	T737C	I51T
HSD11B1	9	B-hydroxysteroid dehydrogenase	AF414124	G446C	Q123H
LDHA	2	lactate dehydrogenase	AJ557233	G46T	silent
LDLRRP1	8	low density lipoprotein receptor related protein 1	AF526393	A459G	3'UTR

Table 1 – contd.

1	2	3	4	5	6
LEPR	6	leptin receptor	AF184173	C609T	T69M
HSL	6	hormone sensitive lipase	AJ006076	G3436T	E263D
LPL	14	lipoprotein lipase	AY332511	G1026A	intron 6
LXRB	6	liver X receptor beta NR1H1	DQ060239	C147T	silent
MC4R	1	melanocortin 4 receptor	AF087937	G678A	D298N
MC5R	6	melanocortin 5 receptor	AF133793	G303A	A109T
MEF2A	1	myocyte enhancer factor 2A	AF053924	G413T	silent
MYF5	5	myogenic factor 5	Y17154	C580T	5' flanking
MYF6	5	myogenic factor 6, herculin	AY327443	T255C	5' flanking
MYH4	12	myosin heavy chain 2D	AJ493461	T26A	3'UTR
MYOD1	2	myogenic factor 3, MYF3	U12574	G566C	R76P
MYOP	14	myopalladin	AJ560657	G298T	3'UTR
PKLR	4	pyruvate kinase	AJ251197	T384C	intron 10
PKM2	7	pyruvate kinase 2 muscle	AJ557235	T32C	3'UTR
PPARG	13	peroxisome proliferator activated receptor gamma 1	AY044238	A324G	promoter
PPARGC1	8	peroxisome proliferator activated receptor gamma coactivator 1	AY484500	T678A	C430A
PRKAG3	15	AMP activated protein kinase γ subunit	AF214521	G1849A	R250Q
PRLR	16	prolactin receptor	U96306	A201G	S591G
QTL BAMHI	X	QTL RFLP marker	AY574041	C94T	marker
RYR1	6	ryanodine receptor	X68247	C1666T	R615C
SFRS1	17	splicing factor arginine/serine rich 1	DQ098951	C1146T	intron
SULT1A1	3	phenol sulfating phenol sulfotransferase 1	AJ885177	G76A	Nd
TGFB1	6	transforming growth factor beta	AJ621785	G180A	intron 6
TGFB1R	1	transforming growth factor receptor beta	AB182258	C141T	P8S
TNNT3	2	skeletal muscle troponin T3	AJ566367	T153C	intron14
TYR	9	tyrosinase	AB207236	C663T	silent

Table 2. Differences in allele frequencies between Polish and Danish Duroc fatteners

SNP symbol	Nucleotide exchange	Allele frequency in Polish Duroc					Allele frequency in Danish Duroc					Significance level
		Allele 1	Allele 2	No. of genotypes			Allele 1	Allele 2	No. of genotypes			
				11	12	22			11	12	22	
1	2	3	4	5	6	7	8	9	10	11	12	13
ACSL	G2645A	0.03	0.97	1	0	28	0.03	0.97	1	0	33	
ADP	G1719A	1.00	0.00	36	0	0	0.90	0.10	32	8	0	xx
CAST	A408G	0.40	0.60	8	13	15	0.21	0.79	3	11	26	
CAST	A47G	0.58	0.42	21	0	15	0.35	0.65	14	0	26	x
CAST	A499C	0.80	0.20	18	9	1	0.29	0.71	5	9	19	xx
CRH	C233T	0.19	0.81	0	14	22	0.34	0.66	2	23	15	xx
CTSB	C162T	0.96	0.04	34	1	1	1.00	0.00	40	0	0	
CYP21	A2991C	0.16	0.84	1	9	25	1.00	0.00	40	0	0	x
CYP2E1	C2412T	1.00	0.00	36	0	0	0.38	0.63	3	24	13	xx
CYP2E1	G744A	1.00	0.00	36	0	0	0.37	0.63	3	24	13	xx
DECR1	G90C	0.51	0.49	7	21	8	0.00	1.00	40	0	0	xx
DES	C749T	1.00	0.00	36	0	0	1.00	0.00	40	0	0	
ESR1	T472C	1.00	0.00	36	0	0	1.00	0.00	40	0	0	
ESR2	G388A	0.99	0.01	35	1	0	1.00	0.00	40	0	0	
FHL3	G312A	1.00	0.00	36	0	0	1.00	0.00	40	0	0	
GAA	T38C	1.00	0.00	36	0	0	0.88	0.12	32	6	2	x
GH	G200T	1.00	0.00	36	0	0	1.00	0.00	40	0	0	
GH	A306T	0.32	0.68	0	23	13	0.59	0.41	13	21	6	xx
GH	A485G	0.07	0.93	1	3	32	0.51	0.49	8	25	7	xx

Table 2 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13
GHR	A155G	0.00	1.00	0	0	36	0.00	1.00	0	0	40	
GYS1	G418A	0.46	0.54	6	21	9	0.33	0.67	5	16	19	
H-FABP	T1324C(Tdel)	0.24	0.76	2	13	21	0.78	0.22	22	18	0	xx
H-FABP	T737C	0.94	0.06	32	4	0	0.91	0.09	33	7	0	
HSD11B1	G446C	0.00	1.00	0	0	36	0.00	1.00	0	0	40	
LDHA	G46T	0.60	0.40	10	23	3	0.54	0.46	13	17	10	xx
LDLRRP1	A459G	0.25	0.75	1	16	19	0.13	0.87	0	10	28	
LEPR	C609T	0.11	0.89	1	6	29	0.00	1.00	0	0	40	x
HSL	G3436T	0.56	0.44	10	20	6	0.79	0.21	26	11	3	xx
LPL	A1026G	0.39	0.61	5	18	13	0.20	0.80	4	8	28	xx
LXRB	C147T	0.56	0.44	10	20	6	0.68	0.33	19	16	5	
MC4R	G678A	0.38	0.62	3	21	12	0.03	0.97	0	2	38	xx
MC5R	A303G	0.76	0.24	19	17	0	0.54	0.46	10	23	7	xx
MEF2A	G413A	0.90	0.10	29	7	0	0.64	0.36	16	19	5	xx
MYF5	C580T	0.99	0.01	35	1	0	1.00	0.00	40	0	0	
MYF6	T255C	0.31	0.69	2	18	15	0.38	0.62	3	24	13	
MYH4	T26A	0.00	1.00	0	0	36	0.00	1.00	0	0	40	
MYOD1	G566C	0.81	0.19	22	13	0	0.40	0.60	10	12	18	xx
MYOP	G298T	0.04	0.96	1	1	34	0.56	0.44	13	19	8	xx
PKLR	C384T	0.58	0.42	16	10	10	0.23	0.78	1	16	23	xx
PKM2	T32C	0.78	0.22	21	14	1	0.21	0.79	0	17	23	xx
PPARG	A324G	0.74	0.26	18	17	1	0.01	0.99	0	1	39	xx
PPARGC1	T678A	0.32	0.68	3	17	16	0.30	0.70	4	16	20	

PRKAG3	G1845A	0.90	0.10	29	7	0	0.76	0.24	22	17	1	
PRLR	A201G	0.65	0.35	15	17	4	0.51	0.49	7	27	6	xx
BamHI	C94T	0.97	0.03	34	2	0	0.93	0.08	34	6	0	
RYR1	C1666T	1.00	0.00	36	0	0	1.00	0.00	40	0	0	
SFRS1	C1146T	0.31	0.69	1	20	15	0.54	0.46	11	21	8	xx
SULT1A1	G76A	0.03	0.97	1	0	35	0.49	0.51	9	21	10	xx
TGFB1	G180A	0.53	0.47	8	17	6	0.79	0.21	26	10	3	xx
TGFB1R	C141T	0.39	0.61	4	20	12	0.18	0.82	0	13	23	xx
TNNT3	T153C	0.07	0.93	1	3	30	0.91	0.19	32	7	0	xx
TYR	T663C	0.79	0.21	21	15	0	0.10	0.90	0	8	32	xx

SNPs showing adverse trends in allele frequencies are marked by bold letters.
Allele 1 refers to nucleotide on the left site of SNP position in column "Nucleotide exchange", e.g. DECR1 G90C, Allele 1 is G, and Allele 2 is C.
Significance level: x – $P<0.05$, xx – $P<0.01$.

Discussion

Following the common opinion that Danish Duroc are more improved than local Duroc populations, one can assume that alleles more frequently occurring in the Danish population could indicate genes responsible for better meat yield and quality. Our results indicate 17 such markers. There are almost no papers comparing SNP frequency identified in pigs belonging to the same breed of different national origin. Some of the associations shown in Table 2 can be confirmed by our earlier findings. DECR1 (2,4-dieonyl CoA reductase 1) enzyme plays a key role in β-oxidation of the fatty acids, and genetic variations within this gene may affect fatty acids composition, especially linoleic acid content and meat quality. DECR1 in the Danish population is monomoprohic (only CC genotype) and this genotype has been proved to be significantly associated with daily gains (Kamiński et al., 2009 a) and, as a member of two SNP haplotypes, with isocitrate dehydrogenase activity and selected meat quality traits: *longissimus thoracis* pH, lightness and redness (Amills et al., 2005). Also in our recent work on additive effect of the same collection of SNPs on growth rate, meat content and selection index (Kamiński et al., 2009 b), positive effects of 3 loci (CYP2E1, PKLR and TNNT3) and another 2 loci (CAST A499C and TYR) were shown to improve meat content and growth rate, respectively. Hernández-Sánchez et al. (2003) showed that MC4R allele G (coding aspartic acid) is associated with higher test and lifetime daily gain and backfat depth at the 10th rib. Advantageous alleles of all the above mentioned loci were significantly more frequent in Danish Duroc fatteners (Table 2).

Our observations presented in this paper open the intriguing question of whether alleles more frequently represented in Danish Duroc participate in phenotypic variation of meat traits (as causal mutations or markers), and whether giving preference to these alleles will accelerate genetic progress in local Duroc population. We are aware that a reliable answer to this question needs testing a wider population of Duroc pigs to confirm significance of chi-square calculations as well as associations studies between selected SNPs and meat yield and quality. Also, deeper functional analysis would be interesting to find out whether the selected SNPs change the level of mRNA, the quantity (or properties) of encoded protein and in effect the value of economically important traits.

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Accepted for printing 15 II 2010

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Zestaw polimorfizmów kandydujących zidentyfikowanych za pomocą mikromacierzy 52 SNP w dwóch subpopulacjach Duroca wykazujący istotne różnice w dystrybucji alleli SNP

STRESZCZENIE

Chociaż każda rasa charakteryzuje się zestawem utrwalonych cech, strategie hodowlane realizowane przez programy narodowe lub firmy komercyjne powodują, że zwierzęta tej samej rasy mogą różnić się istotnie z powodu odrębnego pochodzenia lub innej strategii hodowlanej. Powszechnie wiadomo, że świnie rasy Duroc importowane z Danii do Polski, w porównaniu do świń tej samej rasy wyhodowanych w Polsce różnią się istotnie pod względem cech jakości mięsa. W naszej pracy podjęto próbę sprawdzenia, czy reprezentanci tych dwóch subpopulacji rasy Duroc różnią się istotnie w rozkładzie alleli 52 SNPs zlokalizowanych w 46 genach wybranych z literatury jako potencjalnie wpływających na cechy wieprzowiny. Używając testu chi kwadrat dowiedziono, że 27 SNPs wykazuje istotnie różną dystrybucję alleli. Wśród nich 17 loci wykazało odwrotny trend frekwencji alleli, to znaczy, że allel 1 był częstszy w jednej grupie Duroc, a rzadszy w drugiej grupie i że ta różnica była istotna na poziomie $P<0,01$. Loci te mogą być rozpatrywane jako obiecujące geny kandydujące zaangażowane w kształtowanie cech jakości wieprzowiny.