A subset of candidate polymorphisms identified by 52 SNPs mini-array SNiPORK in two Duroc populations (differed by meat yield and quality) revealed significant differences in SNP allele distributions

Oleński K.[1], Sieczkowska H.[2], Koćwin-Podsiadła M.[2], Help H.[3], Kaminski S.[1]

[1]University of Warmia and Mazury in Olsztyn, Department of Animal Genetics, Poland
[2]University of Podlasie in Siedlce, Department of Pig Breeding and Meat Science, Poland
[3]AsperBiotech, Tartu, Estonia

INTRODUCTION

Although each breed is characterized by a set of established traits/qualities, in breeding strategies led by national programs or commercial companies animals belong to the same breed may differ significantly. We hypothesized that these phenotyping differences should be accompanied by SNPs allele distributions. SNPs showing the most significant differences in allele frequencies could be promising candidate markers useful in marker assisted selection. The aim of this study was to check whether allele frequency of 52 SNPs identified by mini-array SNiPORK differ significantly between two populations of Duroc fatteners.

MATERIALS AND METHODS

78 Duroc unrelated fatteners (38 Danish and 40 Polish) were included into the study. They were fed by the same diet and slaughtered at average weight 76,5 kg. Blood samples were taken to isolate genomic DNA by MasterPure Geneomic Purification Kit (Epicentre). All animals were genotyped for 52 SNPs by the SNiPORK miniarray based on Arrayed Primer Extention (APEX) technology (Fig. 1) (Kaminski et al. 2008). Significance of allele frequency differences were evaluated by chi-square test. Meat parameters were measured and calculated following obligatory and standardized instructions controlled by the government stations responsible for breeding programs in Poland.

RESULTS AND DISCUSSION

Comparing two Duroc population (imported from Denmark, and local Polish) we found a couple of significant differences in meat yield and quality evaluations. Among 10 compared traits, Danish Duroc have always significantly better values, especially for meat content, ham weight, eye muscle area, and pH45 (data not shown). We were inquired whether these phenotyping differences within the same breed kept in the same feeding conditions are correlated by allele distributions in 52 SNPs chosen from literature as potentially influencing meat yield and quality (Brym & Kaminski, 2006).

Using chi square test we found out that 27 SNPs have significantly (p<0,01) different allele distributions. Among them 17 showed adverse trend of allele frequency (marked in table 2 in yellow), it means that allele 1 was major in one Duroc population and minor in another Duroc population and this difference was significant at least on p<0,01.

In several cases the differences were extreme, for example for TNNT2 (skeletal muscle troponin, allele 1 - 0,07 vs 0,91) or PPARG (peroxisome proliferator activated receptor gamma, allele 1 – 0,74 vs 0,01). Several SNP turned to be monomorphic in one population and polymorphic in another, e.g. CYP21, CYP2E1 and DECR1.

The question is - whether these selected SNPs participate in phenotypic variation of meat traits or are their markers? The answer could be positive because random sampling of pigs within two Duroc groups minimized the influence of parent alleles on total allele frequencies. Especially, a handful of SNPs (table 2, marked in yellow) might be considered as potentially useful in practical breeding (mating sows and boars preferring alleles occurred more frequently in Danish Duroc). It probably could accelerate genetic progress in local Duroc population. Before that however, testing wider population of Duroc pigs is necessary to confirm significance of chi-square calculations. 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.

Table 1. Molecular definition of 52 SNiPORK SNPs.

Locus symbol	SSC	Locus name	GenBank	SNP position	SNP significance
ACSL	X	acylo 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 hormon	AF440229	G400A	R28Q
CSTB	13	Cystatin	AJ315561	A367G	D63N
CYP21	7	Steroid 21 hydroxylase	M83939	A2991C	intron splicing site
CYP2E1 C2412T	14	cytochrome p450 2E1	AJ697882	C2412T	5'-flanking
CYP2E1 G744A	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-hydroksysteroid dehydrogenase	AF414124	G446C	Q123H
LDHA	2	Lactate dehydrogenase	AJ557233	G46T	silent
LDLRRP1	8	low density lipoprotein receptor related protein 1	AF526393	A459G	3'UTR
LEPR	6	leptine 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 coactivator1	AY484500	T678A	C430A
PRKAG3	15	AMP activated protein kinase 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	Nucleotyde exchange	Allele and genotype frequency in Polish Duroc					Allele and genotype frequency in Danish Duroc					P
		Allele 1	Allele 2	No of genotypes			Allele 1	Allele 2	No of genotypes			
				11	12	22			11	12	22	
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	0,01
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	0,05
CAST	A499C	0,80	0,20	18	9	1	0,29	0,71	5	9	19	0,01
CRH	C233T	0,19	0,81	0	14	22	0,34	0,66	2	23	15	0,01
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	0,05
CYP2E1	C2412T	1,00	0,00	36	0	0	0,38	0,63	3	24	13	0,01
CYP2E1	G744A	1,00	0,00	36	0	0	0,37	0,63	3	24	13	0,01
DECR1	G90C	0,51	0,49	7	21	8	0,00	1,00	40	0	0	0,01
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	0,05
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	0,01
GH	A485G	0,07	0,93	1	3	32	0,51	0,49	8	25	7	0,01
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	0,01
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	0,01
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	0,05
HSL	G3436T	0,56	0,44	10	20	6	0,79	0,21	26	11	3	0,01
LPL	A1026G	0,39	0,61	5	18	13	0,20	0,80	4	8	28	0,01
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	0,01
MC5R	A303G	0,76	0,24	19	17	0	0,54	0,46	10	23	7	0,01
MEF2A	G413A	0,90	0,10	29	7	0	0,64	0,36	16	19	5	0,01
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	0,01
MYOP	G298T	0,04	0,96	1	1	34	0,56	0,44	13	19	8	0,01
PKLR	C384T	0,58	0,42	16	10	10	0,23	0,78	1	16	23	0,01
PKM2	T32C	0,78	0,22	21	14	1	0,21	0,79	0	17	23	0,01
PPARG	A324G	0,74	0,26	18	17	1	0,01	0,99	0	1	39	0,01
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	0,01
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	0,01
SULT1A1	G76A	0,03	0,97	1	0	35	0,49	0,51	9	21	10	0,01
TGFB1	G180A	0,53	0,47	8	17	6	0,79	0,21	26	10	3	0,01
TGFB1R	C141T	0,39	0,61	4	20	12	0,18	0,82	0	13	23	0,01
TNNT3	T153C	0,07	0,93	1	3	30	0,91	0,19	32	7	0	0,01
TYR	T663C	0,79	0,21	21	15	0	0,10	0,90	0	8	32	0,01

SNPs showing adverse trends in allele frequencies are marked in yellow.
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.

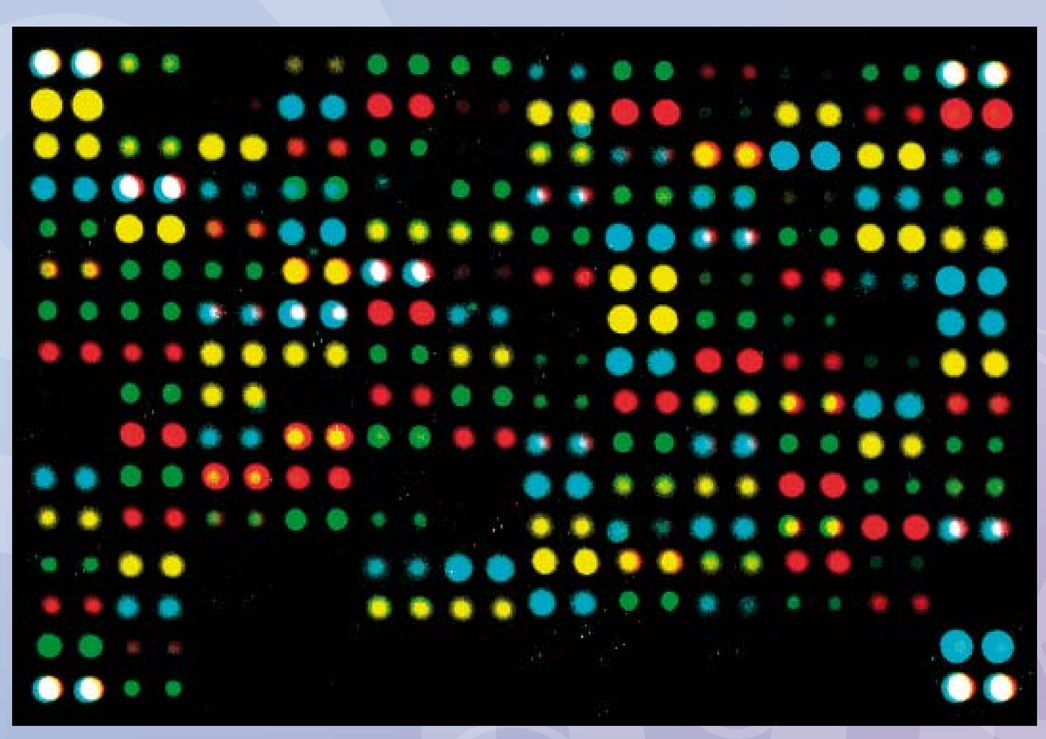

Figure 1. Image of SNiPORK mini-array capable to genotype 52 candidate SNPs.
Colour point indicate nucleotide signals (A-yellow, C-red, G-green, T-cyan).
For detailed description see paper of Kaminski et. al (2008)

ABSTRACT

Although each breed is characterized by a set of established traits and qualities, in breeding strategies led by national programs or commercial companies animals belong to the same breed may differ significantly because of different origin and breeding strategy. Comparing Duroc fatteners imported to Poland from Danish breeding companies versus Duroc pigs kept in Poland (based of import from different countries) we observed significant differences in meat yield and quality parameters. In this report we try to check whether these phenotyping differences are accompanied by allele distributions in 52 SNPs chosen from literature as potentially influencing meat yield and quality. Using chi square test we have found out that 27 SNPs have significantly (p<0,01) different allele distributions. Among them 17 showed adverse trend of allele frequency.

The question is - whether these selected SNPs participate in phenotypic variation of meat traits? If wider population study will confirm this hypothesis, functional analysis is needed to find out whether these 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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ACKNOWLEDGMENTS

This work was supported financially by UWM grant no 0105-804