Associations of fifty single nucleotide polymorphisms within candidate genes with meatness in pigs

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ABSTRACT: The objective of the paper was to classify 50 SNPs (from 17 chromosomes) according to their contribution to the meatness of 293 boars of two breeds (Polish Landrace and Polish Large White) using entropy analysis and standard association analysis. The collected data were classified into two groups (according to the official EUROP procedure) and used for entropy analysis. Associations of single genotypes versus their groups (located at single chromosomes) with the trait studied were estimated by the use of the Generalized Linear Model (GLM). Thus meatness was included as a continuous variable. The most important contributions have been estimated by both approaches for the following SNPs: *SULT1A1:g.76G>A* (SSC3), *PKLR:g.384C>T* (SSC4), *MYOD1:c.566G>C* (SSC2), *TNNT3:g.153T>C* (SSC2), *GAA:g.38T>C* (SSC12), *LDLRR1:c.459A>G* (SSC8), *MYF6:g.255T>C* (SSC5), *CAS:g.499A>C* (SSC2), *PPARGC:c.678T>A* (SSC15). Moreover, interactions among some studied loci are suggested, especially for the loci at chromosome 1.

Keywords: entropy analysis; pork; meat content; SNP

INTRODUCTION

Meatness is one of the main selection criteria in pigs and can be regarded as a complex trait influenced by genetic and environmental factors. Over the last decades many efforts have been focused on detection of single loci underlying meatness. Molecular technology advances have enabled the identification of many chromosomal regions (represented by single nucleotide polymorphisms (SNPs)) affecting swine meat traits (Dekkers et al., 2011). Their effects vary across population and are dependent on data structure, statistical models, and estimation methods. It should be noted that a majority of statistical analyses concerns the association of single polymorphic locus with performance traits. However, a uni-locus analysis can lead to misestimation of genotype effects, therefore taking into account gene interaction is highly desirable. From the point of view of inference effectiveness, evaluation of QTL effects based on crossbreeding experiments is preferable (Dvořáková et al., 2011). Over the last decades, several statistical experimental designs based on crosses have been described in the literature (e.g. Haley and Knott, 1992). Due to a number of polymorphic loci, they have numerous advantages. Unfortunately, from a practical perspective, the estimates of QTL effects cannot be directly transferred into pure breeds and commercial populations. Other approaches to estimate single locus effects are based on field collected data (Janss et al., 1997). However, inclusion of many genotypic effects in a linear model requires a large population with a balanced structure.

Meatness can be considered as a continuous character (expressed in percentages) or a discrete

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one when the so-called EUROP classification is employed. In consequence, this trait can be treated as categorical. It has a complex genetic background (Hamill et al., 2012). Statistical analysis of discrete variables requires other approaches compared to those addressed for continuous characters. One of these methods is the so-called entropy analysis which enables reduction of recorded categorical variables (e.g. genotypes) to their contribution to the final assessment (meatness classes). This methodology has been increasingly implemented in genetic studies (Moore et al., 2006). However, the application of entropy analysis is still marginal in animal science.

The objective of this paper was to classify the effects of fifty candidate single nucleotide polymorphisms (located on 17 chromosomes) according to their contribution in swine meatness. In a second step, association analysis among single genotypes versus their groups (located at single chromosomes) and the trait studied was performed.

MATERIAL AND METHODS

The records of 293 live boars of two breeds (101 – Polish Landrace and 192 – Polish Large White), under performance test, were included into the analysis. A single record has the following structure: recorded animal code, sire code (13), breed (2), year of birth (5), month of birth (12), and meatness (expressed in percentage) as well as polymorphism at 50 loci from 17 chromosomes. Meatness (MC) was measured between days 170–210 of a pig's life and in Poland it is estimated as follows:

$$MC = -0.4776P_{2ST} - 0.4593\ P_{4ST} + 0.3486\ P_4M_{ST} + 48.9829$$

where:

P_{2ST} = standardized backfat thickness at point P2 (behind the last rib, 3 cm from the middle line of the back)

P_{4ST} = standardized backfat thickness at point P4 (behind the last rib, 8 cm from the middle line of the back)

P_4M_{ST} = height of loin at point P4

Average meatness was 59.23 ± 1.45%. All loci studied in the present work were considered as candidate ones, potentially associated with pork carcass quality (Kaminski et al., 2008).

For entropy analysis the meatness was classified according to the so-called EUROP applied by routine carcass classification. Although the classification covers six classes (S = at least 60% meat content, E = 55–60%, U = 50–55%, R = 45–50%, O = 40–45%, and P = less than 40%), the individuals recorded were assigned to groups S (83 records) and E (211 records). In the case of association analysis, meatness was treated as continuous. To improve data structure, some restrictions were done prior to the analysis. The number of individuals per half-sib group and single genotype was at least five. It should be stressed that all experimental animals were free from recessive mutation in *RYR1* gene (Ryanodine Receptor) known to affect the meatness and meat quality. All fatteners were genotyped in 50 loci (Table 1) by the method described earlier (Kamiński et al., 2008). Whereas allelic frequencies in the loci studied for both breeds are listed in Table 2.

Statistical analysis. The effects of the loci studied on meatness were examined in two steps. Firstly, genotypes were classified according to their participation in meatness variability. In the second stage, effects of a single genotype and combined genotypes on the trait recorded were checked.

As already mentioned, 50 identified genotypes were included. In order to rank the loci according to their effects on meatness and relationships among these SNPs, entropy analysis was employed (see e.g. Moore et al., 2006). For each SNP and SNP groups at the same chromosomes entropy and conditional entropy were estimated.

Conditional entropy $H(M|S_i)$ quantifies the remaining uncertainty about meatness (M) with the knowledge of SNP (S_i).

$$H(M|S_i) = -\sum_b p(s_i) \sum_a p(m|s_i) \log p(m|s_i)$$

where:

M = discrete random variables of meatness classes

S_i = discrete random variables of SNP genotype

$p(s_i)$ = probability of a given S_i value

$p(m|s_i)$ = value of conditional probability distributions

For each pair of SNPs, the joint entropy $H(S_i,S_j)$, mutual information $I(S_i,S_j)$, and their quotient were assessed to estimate the interaction between two variables (genotypes). $I(S_i,S_j)$ is a measure of correlation between attributes, which is always zero or positive. It is zero if and only if the two attributes are independent.

More detailed description of the parameters was given by Dobek et al. (2012). The categorization of SNPs to the meatness is shown in Figure 1. The

Table 1. Molecular description of SNPs genotyped in analyzed fatteners (subset of 50 SNPs included in SNiPORK chip, Kamiński et al., 2008)

Gene	Gene name	GenBank accession No.	Locus	SNP function
ACSL4	acylo CoA synthase long chain 4	DQ144454	c.*2645G>A	3'UTR
ADIPOQ	adiponectin C1Q	AJ849536	g.1719G>A	p.Val60Ile
APOA2	apolipoprotein A2	AJ564196	g.350G>A	intron 3
CAST	calpastatin	DQ339697 + AY594692	c.408A>G	p.Asn167Ser
CAST	calpastatin	DD217638	g.47A>G	p.Arg339Lys
CAST	calpastatin	DD217639	g.499A>C	p.Arg728Ser
CRH	corticotropin releasing hormone	AF440229	c.400G>A	p.Arg28Gln
CSTB	cystatin B	AJ315561	g.367A>G	p.Asp63Asn
CYP2E1	cytochrome p 450 2E1	AJ697882	g.2412C>T	5' flanking
CYP2E1	cytochrome p 450 2E1	AJ697884	c.744G>A	p.Ala475Thr
CYP21	steroid 21 hydroxylase	M83939	g.2991A>C	intron splicing site
DECR1	mitochondrial 2,4 dienoyl CoA reductase 1	AF335499	c.90G>C	p.Val54Leu
DES	desmin	AF136188	c.749C>T	silent
ESR1	estrogen receptor 1	AF034974	c.472T>C	silent
ESR2	estrogen receptor 2	AY357117	c.388G>A	p.Met317Val
GAA	alpha acid glucosidase	AJ557226	g.38C>T	silent
GAD2	glutamate decarboxylase 2 gene	AF473817	c.340T>C	intron
GH	growth hormone	U58113	g.200G>T	SP1 binding
GH	growth hormone	U58113	g.306A>T	TATA box
GH	growth hormone	AY727040	c.485A.G	p.Arg22Gln
GHR	growth hormone receptor	DQ388035	c.155A>G	silent
GYS1	glycogen synthase 1	AJ507152	g.418G>A	intron 14
H FABP	heart fatty acid binding protein	X98558	g.1324T>C	5' flanking
HSD11B1	hydroxysteroid (11-beta) dehydrogenase 1	AF414124	c.446G>C	p.Gln123His
LDLRRP1	low density lipoprotein receptor related protein 1	AF526393	c.*459A>G	3'UTR
LEPR	leptine receptor	AF184173	c.609C>T	p.Thr69Met
LPL	lipoprotein lipase	AY332511	g.1026G>A	intron 6
MC4R	melanocortin 4 receptor	AF087937	c.678G>A	p.Asp298Asn
MC5R	melanocortin 5 receptor	AF133793	c.303G>A	p.Ala109Thr
MEF2A	myocyte enhancer factor 2A	AF053924	c.413G>T	silent
MEF2D	myocyte enhancer factor 2D	AJ519842	g.638C>T	intron 4
MYF5	myogenic factor 5	Y17154	g.580C>T	5' flanking
MYF6	myogenic factor 6, herculin	AY327443	g.255T>C	5' flanking
MYH4	myosin heavy chain 4	AJ493461	g.26T>A	3'UTR
MYOD1	myogenic differentiation	U12574	c.566G>C	p.Arg76Pro
MYOG	myogenin	X89007	g.673C>T	silent
MYOP	myopalladin	AJ560657	g.298G>T	3'UTR
PKLR	pyruvate kinase, liver, RBC	AJ251197	g.384T>C	intron 10
PKM	pyruvate kinase, muscle	AJ557235	g.32T>C	3'UTR
PPARG	peroxisome proliferator activated receptor gamma 1	AY044238	g.324A>G	promotor
PPARGC	peroxisome proliferator activated receptor gamma coactivator 1	AY484500	c.678T>A	p.Cys430Ala

Table 1 to be continued

Gene	Gene name	GenBank accession No.	Locus	SNP function
PRKAG3	protein kinase, AMP activated, gamma subunit	AF214521	c.1845G>A	p.Val249Ile
PRLR	prolactin receptor	U96306	c.201A>G	p.Ser591Gly
QTL BamHI	QTL RFLP marker	AY574041	g.94C>T	marker
SFRS1	splicing factor arginine/serine rich 1	DQ098951	c.1146C>T	intron
SULT1A1	phenol preferring sulfotransferase 1	AJ885177	g.76G>A	nd
TGFB1	transforming growth factor β1	AJ621785	g.180G>A	intron 6
TGFB1R	transforming growth factor β1 receptor	AB182258	c.141C>T	p.Pro8Ser
TNNT3	troponin T, type 3	AJ566367	g.153T>C	intron 14
TYR	tyrosinase	AB207236	c.663C>T	silent

*substitution located in the 3'UTR, nd = no data

graphs show the following values: $H(M) - H(M|S_i)$, where predicted variable M is meatness and S_i denotes genotypes. To visualize relationships among the recorded loci, they were clustered using the method of hierarchical clustering according to the Ward approach (Jobson, 1992). Constructed dendrogram shows related loci close together. The reciprocity of normed mutual information, i.e. $H(S_i,S_j)/I(S_i,S_j)$, was used as the distance measure. Analyses were performed using the R software package (Version 2.1.0, 2009).

Prior to association studies, exploratory analysis was performed to improve the data structure. Significance of breed, sire, month, and year of birth were evaluated using the Analysis of Variance (ANOVA) procedure of SAS (Statistical Analysis System, Version 9.1, 2002–2003). Based on these results, only birth year and sire effects were included into the association analysis as fixed and random, respectively. Finally, the following linear model was used:

$$y = \mathbf{X}_1\beta_1 + \mathbf{X}_2\beta_2 + \mathbf{Z}u + e$$

where:

y = vector of observation for meatness (as a continuous variable)

β_1 = vector of fixed genotypic or chromosomal effects (groups with at least 5 observations were analyzed)

β_2 = vector of fixed effects of birth years

u = vector of random sire effects

e = vector of random residuals

$\mathbf{X}_1$, $\mathbf{X}_2$, $\mathbf{Z}$ = respective incidence matrices for fixed and respective effects

Statistical inference on differences among these genotypic means was based on F-test using the above mentioned procedure of SAS.

RESULTS AND DISCUSSION

Classification of loci and chromosomes. Conditional entropy coefficients for single loci and their groups (at the same chromosome) are visualized in Figures 1 and 2, respectively. As already mentioned, the fifty loci analyzed were perceived as candidate genes influencing pig meatness. However, participation of particular loci in the studied trait varied. Although these entropy coefficients cannot be statistically verified, some considerable differences between loci contribution to meatness were observed. For eleven loci the entropies were higher than 0.01. Most important contributions have been estimated for SNPs: *SULT1A1:g.76G>A* (SSC3), *PRKAG3:c.1845G>A* (SSC15), *PKLR:g.384T>C* (SSC4), *ADPIPOQ:g.1719G>A* (SSC13), *MYOD1:c.566G>C* (SSC2), and *TNNT3:g.153T>C* (SSC2). Unfortunately, the number of reports on the effects of the above mentioned molecular regions is still relatively small. The majority of these analyses focus on estimation of single locus effects. Moreover, many studies are based on crossbreeding experiments. Hence, direct comparison of the obtained results with literature reports seems to be difficult. The highest participation in meatness was estimated for *SULT1A1:g.76G>A*. Unfortunately, to our knowledge, association of *SULT1A1* gene with swine production traits has not yet been sufficiently described. Skinner et al. (2006) suggested potential associations of the gene with swine skatole levels, however this trait is not directly connected with meatness. This locus has already been recommended as potentially associated with pork traits by Kamiński et al. (2009).

Many reports concern porcine *PRKAG3* gene as a gene influencing meatness (Škrlep et al., 2010a; Rohrer et al., 2012). Qiao et al. (2010) showed a

Table 2. Frequency of alleles in loci studied across two breeds

Gene	Locus	Allele	Frequency		Gene	Locus	Allele	Frequency	
			PL	PLW				PL	PLW
ACSL	c.*2645G>A	*A*	0.508108	0.040816	*LEPR*	c.609C>T	*C*	0.203125	0.764706
		G	0.491892	0.959184			*T*	0.796875	0.235294
ADIPOQ	g.1719G>A	*A*	0.068783	0	*LPL*	g.1026G>A	*A*	0.624339	0.362745
		G	0.931217	1			*G*	0.375661	0.637255
APOA2	g.350G>A	*A*	0.342932	0.093137	*MC4R*	c.678G>A	*A*	0.236979	0.617647
		G	0.657068	0.906863			*G*	0.763021	0.382353
CAST	c.408A>G	*A*	0.742188	0.529412	*MC5R*	c.303G>A	*A*	0.338542	1
		G	0.257813	0.470588			*G*	0.661458	0
CAST	g.47A>G	*A*	0.863128	0.704545	*MEF2A*	c.413G>T	*A*	0.39267	0.617647
		G	0.136872	0.295455			*G*	0.60733	0.382353
CAST	g.499A>C	*A*	0.824607	0.623762	*MEF2D*	g.638C>T	*C*	0.973404	0.840206
		C	0.175393	0.376238			*T*	0.026596	0.159794
CRH	c.400G>A	*C*	0.619681	0.292079	*MYF5*	g.580C>T	*C*	0.986979	0.745098
		T	0.380319	0.707921			*T*	0.013021	0.254902
CSTB	g.367A>G	*C*	0.612903	0.569307	*MYF6*	g.255T>C	*C*	0.361257	0.29902
		T	0.387097	0.430693			*T*	0.638743	0.70098
CYP2E1	g.2412C>T	*C*	0.376963	0.495098	*MYH4*	g.26T>A	*A*	0.904762	0.855
		T	0.623037	0.504902			*T*	0.095238	0.145
CYP2E1	c.744G>A	*A*	0.622396	0.514706	*MYOD1*	c.566G>C	*C*	0.148649	0.070707
		G	0.377604	0.485294			*G*	0.851351	0.929293
CYP21	g.2991A>C	*A*	0.596354	0.754902	*MYOG*	g.673C>T	*C*	0.966146	0.925743
		C	0.403646	0.245098			*T*	0.033854	0.074257
DECR1	c.90G>C	*C*	0.585938	0.534314	*MYOP*	g.298G>T	*G*	0.218023	0.436275
		G	0.414063	0.465686			*T*	0.781977	0.563725
DES	c.749C>T	*C*	0.863874	0.970588	*PKLR*	g.384T>C	*C*	0.316754	0.436275
		T	0.136126	0.029412			*T*	0.683246	0.563725
ESR1	c.472T>C	*C*	0.125654	0.514851	*PKM*	g.32T>C	*C*	0.453125	0.705882
		T	0.874346	0.485149			*T*	0.546875	0.294118
ESR2	c.388G>A	*A*	0.796875	0.392157	*PPARG*	g.324A>G	*A*	0.473958	0.308824
		G	0.203125	0.607843			*G*	0.526042	0.691176
GAA	g.38C>T	*C*	0.497382	0.292079	*PPARGC*	c.678T>A	*A*	0.481771	0.313725
		T	0.502618	0.707921			*T*	0.518229	0.686275
GAD2	c.340T>C	*C*	0.889474	1	*PRKAG3*	c.1845G>A	*A*	0.632275	0.196078
		T	0.110526	0			*G*	0.367725	0.803922
GH	g.200G>T	*G*	0.994792	0.803922	*PRLR*	c.201A>G	*A*	0.710938	0.460784
		T	0.005208	0.196078			*G*	0.289063	0.539216
GH	g.306A>T	*A*	0.611979	0.382353	*QTL BamHI*	g.94C>T	*C*	0.174479	0.460784
		T	0.388021	0.617647			*T*	0.825521	0.539216
GH	c.485A>G	*A*	0.955497	0.568627	*SFRS1*	c.1146C>T	*C*	0.442408	0.431373
		G	0.044503	0.431373			*T*	0.557592	0.568627
GHR	c.155A>G	*A*	0.502604	0.004902	*SULT1A1*	g.76G>A	*A*	0.719577	0.475248
		G	0.497396	0.995098			*G*	0.280423	0.524752
GYS1	g.418G>A	*A*	0.751309	0.695	*TGFB1*	g.180G>A	*A*	0.093583	0.21
		G	0.248691	0.305			*G*	0.906417	0.79
H FABP	g.1324T>C	*C*	0.052356	0.268421	*TGFB1R*	c.141C>T	*C*	0.459893	0.484848
		T	0.947644	0.731579			*T*	0.540107	0.515152
HSD11B1	c.446G>C	*C*	0.95288	0.519608	*TNNT3*	g.153T>C	*C*	0.847594	0.217822
		G	0.04712	0.480392			*T*	0.152406	0.782178
LDLRRP1	c.*459A>G	*A*	0.223757	0.519608	*TYR*	c.663C>T	*C*	0.476563	0.632353
		G	0.776243	0.480392			*T*	0.523438	0.367647

PL = Polish Landrace, PLW = Polish Large White, *substitution located in the 3'UTR

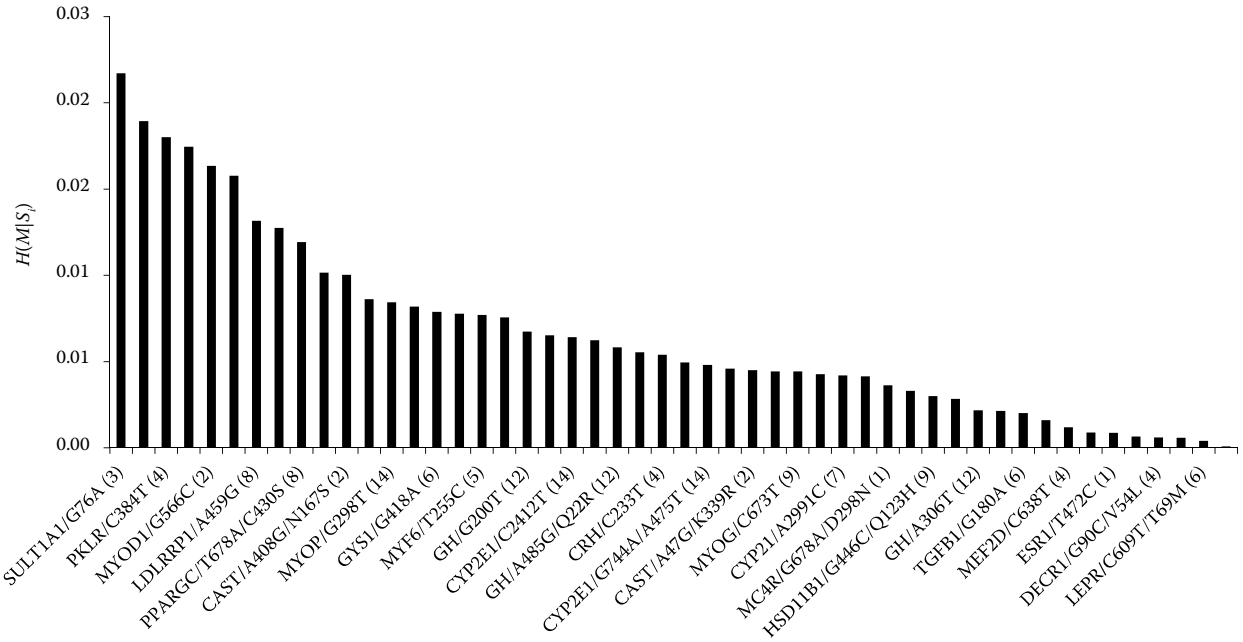

Figure 1. Categorization of loci to meatness
$H(M|S_i)$ = conditional entrophy, number of chromosomes given in parentheses

considerable association between this gene and two meat quality traits (pH and glycogen). By the way, it should be stressed that imprinted action of this gene has been registered by the authors. These results were obtained for crossbreeding populations. Thus, by definition the estimated effects are higher due to greater heterozygosity compared to outbreed genetic groups. The impact of *PRKAG3* was also investigated by Koćwin-Podsiadła et al. (2006). They reported significant association of this locus with the meat production traits.

A majority of the loci studied showed relatively small contributions in meatness. The effects of some of them have been described in literature. For instance, several single nucleotide polymorphisms in *CAST* have been associated with carcass quality (Krzęcio et al., 2007; Lindholm-Perry et al., 2009; Škrlep et al., 2010b)

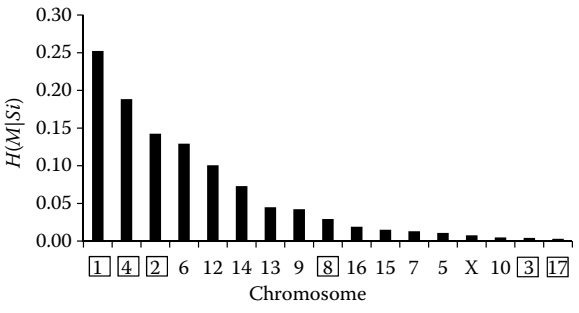

Figure 2. Categorization of chromosomes to meatness
$H(M|S_i)$ = conditional entrophy, □ = chromosomes with the most significant contribution to the trait

Sieczkowska et al. (2010) showed the impact of two genes (*PKM2* and *CAST*) on their expression levels in muscles and some traits. However, the effects were strongly dependent on parental components. A larger one was estimated for the Duroc paternal side, whereas negligible impacts were registered on several genes (located on different chromosomes as well). Significant effect of *CAST* gene on meat quality was also reported by Gandolfi et al. (2011).

Combined effects of SNPs groups from single chromosomes. It can be observed that chromosomal effects depend on a number of identified loci. On the other hand, these effects of particular chromosomes (with the same number of loci) in meatness vary as well. For instance, in the case five chromosomes (1, 4, 2, 6, and 12), five loci per each, were studied and considerable differences among them were observed (Figure 2). The largest effects were observed for chromosome 1 (including *MEF2A:c.413G>T*, *MC4R:c.678G>A*, *ESR2:c.388G>A*, *TGFB1R:c.141C>T*, *ESR1:c.472T>C*). Although single effects of these loci were relatively small, their combined effects were considerably larger compared to hypothetical additive effects of single loci. This indicates that interlocus effects should be considered for chromosome 1 (Figure 2). Also interlocus effects may be suggested for some other

Table 3. Significance of the effects of individual loci on meatness

Locus	Significance level	Locus	Significance level
*ACSL:c.*2645G>A*	0.9183	*LEPR:c.609C>T* (p.Thr69Met)	0.6034
ADIPOQ:g.1719G>A (p.Val60Ile)	0.2429	*LPL:g.1026A>G*	0.3347
APOA2:g.350G>A	0.3723	*MC4R:c.678G>A* (p.Asp298Asn)	0.4341
CAST:c.408A>G (p.Asn167Ser)	0.0619	*MC5R:c.303G>A* (p.Thr109Ala)	0.5719
CAST:g.47A>G (p.Lys339Arg)	0.9029	*MEF2A:c.413G>T*	0.1237
***CAST:g.499A>C* (p.Arg728Ser)**	**0.0219**	*MEF2D:g.638C>T*	0.2821
CRH:c.233C>T (p.Gly400Ala)	0.1979	*MYF5:g.580C>T*	0.3787
CSTB:g.367A>G	**0.0081**	***MYF6:g.255T>C***	**0.0459**
CYP2E1:c.744G>A (p.Ala475Thr)	0.3288	*MYH4:g.26T>A*	0.4704
CYP2E1:g.2412C>T	0.2785	*MYOD1:c.566G>C*	0.0118
CYP21:g.2991A>C	0.0834	*MYOG:g.673C>T*	0.7337
DECR1:c.90G>C (p.Val54Leu)	0.4792	*MYOP:g.298G>T*	0.7615
DES:c.749C>T	0.5247	***PKLR:g.384C>T***	**0.0002**
ESR1:c.472T>C	0.9801	*PKM2:g.32T>C*	0.8552
ESR2:c.388G>A (p.Val317Met)	0.4938	*PPARG:g.324A>G*	0.9761
GAA:g.38T>C	**0.0429**	***PPARGC:c.678T>A* (p.Cys430Ser)**	**0.0053**
GAD2:c.340C>T	0.5756	*PRKAG3:c.1845G>A* (p.Val249Ile)	0.1941
GH:g.306A>T	0.3113	*PRLR:c.201A>G* (p.Ser591Gly)	0.7082
GH:c.485A>G (p.Gln22Arg)	0.6352	*QTL BamHI:g.94C>T*	0.417
GH:g.200G>T	0.3826	***SFRS1:c.1146C>T***	**0.0019**
GHR:c.155A>G	0.9474	***SULT1A1:g.76G>A***	**0.0178**
GYS1:g.418G>A	0.6008	*TGFB1:g.180G>A*	0.0999
H FABP:g.1324T>C	0.0708	*TGFB1R:c.141C>T* (p.Pro8Ser)	0.0750
HSD11B1:c.446G>C (p.Gln123His)	0.8753	***TNNT3:g.153T>C***	**0.0027**
LDLRRP1:c.*459A>G	**0.0207**	*TYR:c.663C>T*	0.7312

Loci in bold have the most significant contribution to the trait, *substitution located in the 3'UTR

chromosomes, mainly chromosomes 2, 4, and 6. The obtained results confirmed the presence of more loci on chromosome 4 affecting fatness and growth (Marklund et al., 1999). Also Ovilo et al. (2002) reported that chromosomes 4 and 6 contain a loci determining meat quality traits. However, they estimated non-significant epistatic interactions for these characters. On the other hand, some authors (Uemoto et al., 2009; Groβe-Brinkhaus et al., 2010) suggested that epistasis might be an important component of production traits (including meatness) in pigs. It should be noted that the above mentioned considerable interlocus effects were estimated for crossbred populations.

The smallest effects were estimated for chromosomes with one identified locus (Figure 2). These effects also vary among chromosomes. They can be directly determined by both genotype frequencies and additive effects of these loci. On the other hand, an interaction among and within loci may also be shown in the ranking of chromosomes. Complex genetic determination of meatness has been reported by a number of authors (Kamiński et al., 2009; Srikanchai et al., 2010; Weisz et al., 2011; Fontanesi et al., 2012).

Relationships among loci. To summarize better the mutual information coefficients, their values were clustered. Figure 3 shows the cluster dendrogram of mutual information coefficients for single loci, which illustrates connectedness among them. These coefficients range from 0 to 1 and show the relationships between pairs of groups and/or single loci. So, strongly related SNPs and/or groups at the same chromosomes, with coefficient of mutual information, are close together on the diagram. The dependencies among studied loci may be influenced by manifold genetic backgrounds, e.g. linkage disequilibrium, meiotic drive, etc. In general, a majority of locus clusters have a mixed composition according to their chromosomal localizations. Also the magnitude of these clusters is

Table 4. Effects of individual chromosomes on meatness

Chromosome		Number of loci studied	Number of combined genotypes	Significance level
No.	loci			
1	***TGFB1R:c.141C>T* (p.Pro8Ser)** ***MEF2A:c.413G>A*** ***ESR1:c.472T>C*** ***MC4R:c.678G>A* (p.Asp298Asn)** ***ESR2:c.388G>A* (p.Val317Met)**	**5**	**17**	**0.0435**
2	***TNNT3:g.153T>C*** ***CAST:c.408A>G* (p.Asn167Ser)** ***CAST:g.47A>G* (p.Lys339Arg)** ***MYOD1:c.566G>C*** ***CAST:g.499A>C* (p.Arg728Ser)**	**5**	**14**	**0.0246**
3	***SULT1A1:g.76G>A***	**1**	**3**	**0.0178**
4	***APOA2:g.350G>A*** ***MEF2D:g.638C>T*** ***PKLR:g.384C>T*** ***DECR1:c.90G>C* (p.Val54Leu)** ***CRH:c.400G>A***	**5**	**24**	**0.0006**
5	*MYF6:g.255T>C* *MYF5:g.580C>T*	2	5	0.1869
6	*GYS1:g.418G>A* *H FABP:g.1324T>C* *LEPR:c.609C>T* (p.Thr69Met) *MC5R:c.303A>G* (p.Thr109Ala) *TGFB1:g.180G>A*	5	21	0.1841
7	*CYP21:g.2991A>C* *PKM:g.32T>C*	2	9	0.1718
8	***LDLRRP1:c.*459A>G*** ***PPARGC:c.678T>A* (p.Cys430Ala)**	**2**	**9**	**0.0047**
9	*MYOG:g.673C>T* *TYR:c.663T>C* *HSD11B1:c.446G>C* (p.Gln123His)	3	8	0.4364
10	*GAD2:c.340C>T*	1	2	0.5756
12	*GAA:g.38T>C* *GH:g.200G>T* *GH:g.306A>T* *GH:c.485A>G* (p.Gln22Arg) *MYH4:g.26T>A*	5	16	0.7873
13	*ADPIPOQ:g.1719G>A* (p.Val60Ile) *CSTB:g.367A>G* *PPARG:g.324A>G*	3	10	0.0727
14	*CYP2E1:g.2412C>T* *CYP2E1:c.744G>A* (p.Ala475Thr) *MYOP:g.298G>T* *LPL:g.1026A>G*	4	19	0.3287

Table 4 to be continued

Chromosome		Number of loci studied	Number of combined genotypes	Significance level
No.	loci			
15	*DES:c.749C>T* *PRKAG3:c.1845G>A* (p.Val249Ile)	2	5	0.0767
16	*PRLR:c.201A>G* (p.Ser591Gly) *GHR:c.155A>G*	2	8	0.3250
17	***SFRS1:c.1146C>T***	**1**	**3**	**0.0019**
X	*QTL BamHI:g.94C>T* *ACSL:c.*2645G>A*	2	4	0.3484

Loci in bold have the most significant contribution to the trait, *substitution located in the 3'UTR

differentiated. It seems that the gene architecture is connected with meatness complexity, which is affected by the muscle structure and composition. As reported by Hu et al. (2005), quantitative trait loci have been detected for the most important traits in pigs, including carcass composition. Moreover, some authors (e.g. Gilbert et al., 2007) concluded pleiotropic and linked QTL effects on porcine carcass.

Estimated single locus and chromosomal effects. Tables 3 and 4 show the results of association analysis for single loci and chromosomal regions, respectively. The performed analysis showed a significant effect of the following ten loci on meatness: *PKLR:g.384T>C, SFRS1:c.1146C>T, PPARGC:c.678T>A, TNNT3:g.153T>C, CSTB:g.367A>G, SULT1A1:g.76G>A, LDLRRP1:c.*459A>G, CAST:g.499A>C, GAA:g.38C>T,* and *MYF6:g.255T>C.*

Out of 17 chromosomes included in the present study, six (1, 2, 3, 4, 7, and 8) were found to affect meatness significantly. It should be noted that this is not directly connected with the number of loci identified at particular chromosomes and their single effects. For instance, in the case of uni-locus association analysis, no statistically significant effects were estimated for each five loci from chromosome 1. It can result from at least two reasons. Firstly, when single genotype effects were examined, statistical inferences were based on a relatively small number of degrees of freedom. Secondly, it can suggest a theoretical large interaction effect among the five loci from chromosome 1. Generally, the results correspond with the ranking of loci performed via conditional entropy (see Figure 1), where the participation of three loci (*ESR1:c.472T>C, ESR2:c.388G>A* (p.Met317Val), *MC4R:c.678G>A* (p.Asp298Asn))

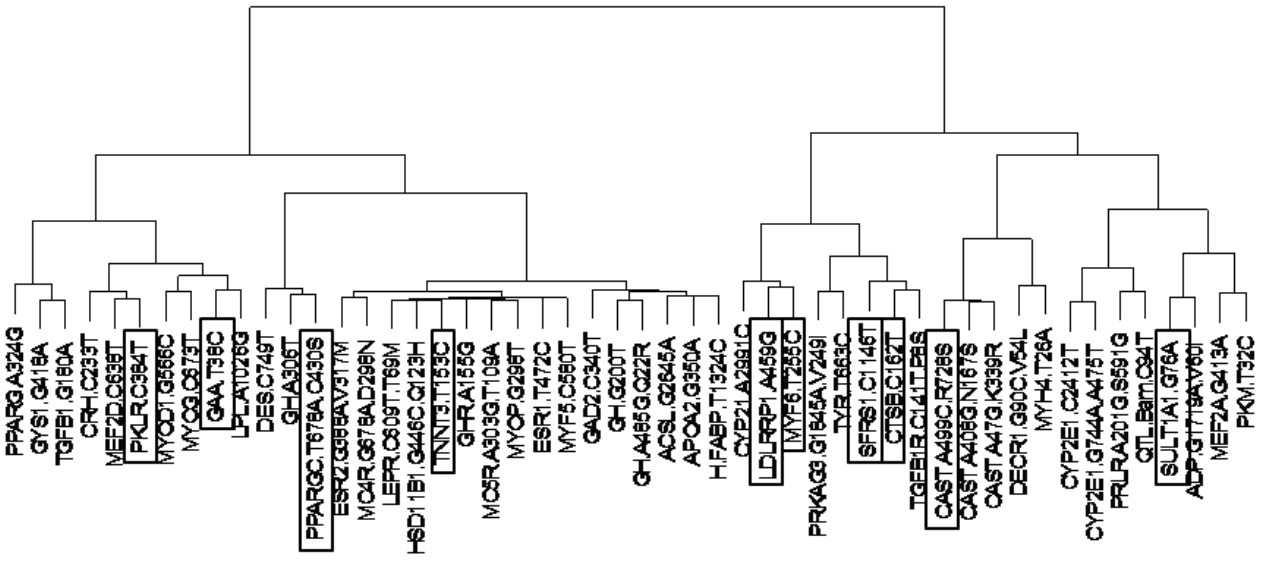

Figure 3. Cluster dendrogram of the analyzed loci

is regarded as negligible. However, in the case of chromosomes 2 and 4, the effects of the five loci identified on each of them varied. Finally, combined influences were considerably larger compared to single ones, whereas for loci from chromosome 8, both single and combined effects were statistically significant. This suggests a large and additive effect of loci *LDLRRP1:c.*459A>G* and *SFRS1:c.1146C>T*.

Joint analyzed results. By definition, an entropy analysis is addressed for discrete variables. Hence, two meatness classes (among five hypothetical ones) were included. It should be noted that statistical inference is based on a linear model, which includes random sire effects as well as fixed genotypic vs. chromosomal and birth year effects. Due to unavailability of a full additive relationship matrix, additive genetic effects of individuals were not included into the analysis. There may be a negligible influence of statistical inference on the significance of genotypic effects since a sire effect is not directly perceived as substitution of an additive polygenic one. Moreover, single genotype vs. single chromosome effects were included into the model. Therefore, the analysis omitted multigenotype effects across chromosome.

CONCLUSION

In the present study, two different statistical approaches were employed to detect loci significantly affecting the meatness of boars. Among 50 SNPs analyzed, the following can be indicated as the most important: *SULT1A1:g.76G>A* (SSC3), *PKLR:g.384T>C* (SSC4), *MYOD1:c.566G>C* (SSC2), *TNNT3:g.153T>C* (SSC2), *GAA:g.38C>T* (SSC12), *LDLRRP1:c.*459A>G* (SSC8), *MYF6:g.255T>C* (SSC5), *CAST:g.499A>C* (SSC2), and *PPARGC:c.678T>A* (SSC15).

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