Polymorphism, linkage mapping, and association analysis with carcass traits of four porcine candidate genes selected from gene-expression profiles of Czech Large White and Wild Boar muscles

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ABSTRACT: Genes that are expressed in skeletal muscles may play a role in prenatal muscle development and postnatal muscle growth and can be considered candidates for economically important traits. Four porcine genes that were differentially expressed in skeletal muscles of Czech Large White and Wild Boar (*SORT1*, *EMP3*, *IL18,* and *BTG2*) were selected to search for polymorphism, linkage assignment, and association analysis with carcass traits. Through comparative sequencing of portions of the genes numerous polymorphisms were revealed (*SORT1* – 21, *EMP3* – 6, *IL18* – 41, *BTG2* – 9). Linkage analysis in a Meishan × Pietrain F_2 pedigree showed the positions of the genes relative to other genes and markers on the respective chromosomes – *SORT1* on SSC4, *EMP3* on SSC6, *IL18* and *BTG2* on SSC9. Preliminary association analysis in pig commercial crosses with selected SNPs showed associations with several carcass traits at nominal *P* value of < 0.05, which may indicate their involvement in muscle growth and fat deposition. The tested polymorphisms may not be causal for the associations, but they may be in linkage disequilibrium with causative mutations.

Keywords: pig; single nucleotide polymorphism; DNA markers; DNA sequencing; PCR-RFLP; *SORT1*; *EMP3*; *IL18*; *BTG2*

Postnatal muscle growth in pigs encompasses both elongation and hypertrophy of prenatally formed muscle fibres and tertiary myofibres that are formed between birth and 28 days of age (Goldspink, 2004; Rehfeldt et al., 2004; Bérard et al., 2011). These processes result from gene transcriptional and regulatory activities. The genes that are expressed in skeletal muscles during prenatal

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and postnatal development can serve as a source of candidate genes to study muscle development, growth, carcass traits, and meat quality. Differences in expression between various pig breeds may indicate genetic differences in gene activities that can be caused by mutations. The search for polymorphisms in such genes and association analysis in suitable populations may reveal mutations which can have either positive or negative effect on the studied traits.

Wimmers et al. (2007) considered ten porcine genes selected from differential gene expression analysis of the prenatal muscle development for association study with meat quality and carcass traits. Polymorphisms were found in the genes, and nine of them showed associations with traits related to meat quality and muscle deposition in commercial purebreds, crossbreds, and an experimental F_2 population. D'Andrea et al. (2011) compared patterns of expression of *m. longissimus dorsi* genes during growth of the autochthonous breed Casertana with those of Large White and a crossbred. A total of 173 genes exhibited significant differences in gene expression at 3 months of age. Of these, 14 genes were proposed to be significant for muscle growth or meat quality.

Great differences were observed in growth and muscle fibre properties between modern pig breeds and Wild Boar. The European Wild Boar has a very limited growth potential (Weiler et al., 1998). The differences between the two types of pigs may be due to different activities of various genes, including those expressed in muscles. Genetic factors play a major role in gene expression differences between divergent types of pigs, and one of these factors may be mutations, either in coding or regulatory regions.

When studying differences in the expression of genes in *m. longissimus lumborum et thoracis* (*m.l.l.t.*) and *m. biceps femoris* of modern pigs (Czech Large White) and European Wild Boar during postnatal growth on the Affymetrix GeneChip Porcine Genome Array (which contains probes representing 20 201 *S. scrofa* genes), more than 500 genes exhibited a more than threefold difference (higher or lower) between Czech Large White and European Wild Boar (Stratil et al., unpublished). Based on the expression differences, as well as considering their potential role in muscle growth and expected locations in QTL intervals of respective chromosomes, four genes were selected (*SORT1*, *EMP3*, *IL18,* and *BTG2*):

SORT1 (sortilin 1) is a 100 kDa protein that belongs to a family of multiligand type-1 membrane receptors with homology to the yeast receptor Vps10p (Nielsen et al., 2001). Sortilin interacts with a wide array of proteins (Kwon and Christian, 2011). It forms a complex with an unprocessed form of nerve growth factor (proNGF) and p75NTR, a low affinity NGF receptor, and triggers p75NTR signalling cascades leading to various cellular responses, including differentiation, survival, and apoptosis (Ariga et al., 2008). mRNA transcripts of human *SORT1* are expressed in high levels in skeletal muscle, as well as in the brain, spinal cord, heart, thyroid, placenta, and testis (Vincent et al., 1999). Ariga et al. (2008) studied the functional role of sortilin in myogenesis in C2C12 myocytes (mouse skeletal muscle cell line) and concluded that it was directly involved in muscle differentiation via modulation of proNGF-p75NTR. Fine tuning of the expression levels of these proteins would be required for the proper development and maintenance of healthy muscle tissue. The QuickGO database (http://www.ebi.ac.uk/QuickGO) lists numerous GO terms including regulation of gene expression, myotube differentiation, and multicellular organismal development.

EMP3 (epithelial membrane protein 3) is a transmembrane signalling molecule which has been implicated in the regulation of cell proliferation and apoptosis. mRNA transcripts of *EMP3* were detected in all adult human tissues examined, including skeletal muscle (Taylor and Suter, 1996). The QuickGO database lists biological processes and components in which EMP3 is involved, including the negative regulation of cell proliferation and cell growth.

IL18 (interleukin 18) plays an important role in cell-mediated immune response. It is produced in many different cell types, including macrophages, endothelial cells, vascular smooth muscle cells, dendritic cells, and Kupffer cells (Gerdes et al., 2002; Trøseid et al., 2010). Moreover, IL18 was found to be expressed also in human muscles, but solely in type II fibres, while IL6 was more prominent in type I fibres. This indicates that differing cytokine expressions in different muscle fibre types may play specific regulatory roles in muscle physiology (Plomgaard et al., 2005). Netea et al. (2006), by studying *Il18* knockout mice, provided evidence for an important role of Il18 in energy and glucose homeostasis. Il18-deficient mice had increased body weight, which

resulted from increased food and energy intake. The AgriGO database (http://bioinfo.cau.edu.cn/agriGO/) reports GO term growth for this gene. Porcine *IL18* was mapped to chromosome 9p13 by fluorescence *in situ* hybridization (Fournout et al., 2000).

BTG2 (BTG family, member 2; PC3) belongs to a family of structurally related proteins with antiproliferative activity and involvement in cell cycle regulation (Montagnoli et al., 1996; Winkler, 2010). Feng et al. (2007) studied mRNA expression levels of porcine *BTG2* and *BTG3* genes in proliferating murine myoblast cells and concluded that the two genes may be important for skeletal muscle growth. *BTG2* is expressed at high levels in porcine foetal and adult skeletal muscle tissue (Feng et al., 2007), and different breeds differ in the expression level – Large White has a higher expression compared to Meishan (Mo et al., 2011). Porcine *BTG2* was mapped by radiation hybrid mapping to SSC9, and the most likely chromosomal location is 9q2.1–q2.5 (Feng et al., 2007).

In these four genes, polymorphism was searched for by comparative sequencing, selected polymorphisms were used to estimate allele frequencies in purebred pigs, to perform linkage mapping in the Hohenheim Meishan × Pietrain F_2 pedigree and to carry out association analysis in commercial crossbreds.

MATERIAL AND METHODS

Animals and analyzed traits. DNA was isolated from blood of three groups of pigs. The first group were unrelated purebred pigs of eight breeds – Czech Large White (CZLW), Czech Landrace (CZL), Czech Meat Pig, Pietrain (PN), Black Pied Prestice, Hampshire, Duroc, and Meishan – and Wild Boar animals (see the Results section). The DNA samples were used for the genotyping of the four genes (*SORT1*, *EMP3*, *IL18,* and *BTG2*) for estimation of allele frequencies. Some samples (CZLW, Meishan, Wild Boar) were used for resequencing. The second group were the pigs of the Hohenheim Meishan × Pietrain three-generation pedigree (n = 347) (Geldermann et al., 2003). The samples were genotyped for linkage mapping. The third group consisted of commercial crossbreds and a purebred (Table 1). These animals were used for a preliminary association analysis of genotypes of the four genes (*SORT1*, *EMP3*, *IL18,* and *BTG2*) with performance traits.

Table 1. Pig crossbreds used for association analysis

Group	Pig crosses	n
1	PN × (CZL × CZLW)	156
2	CZLW	22
3	CZLW × CZL	23
4	CZLW × (CZL × CZLW)	72
5	(CZLW × PN) × (CZL × CZLW)	100
6	(D × CZLW) × (CZL × CZLW)	72
7	DanBred	143
8	FH × PIC	63
9	(H × PN) × (CZL × CZLW)	72
10	PIC × (CZL × CZLW)	34

CZLW = Czech Large White, CZL = Czech Landrace, PN = Pietrain, H = Hampshire, FH = French hybrids, PIC = Pig Improvement Company, D = Duroc

The association analyses were carried out for ten commercial populations (Table 1). CZL and CZLW were used for crossing in seven of the populations; one population was a purebred CZLW. The populations were maintained in air-conditioned stables under conditions described by Dvořáková et al. (2012). The pigs were slaughtered at a target weight of 115 kg (SD ± 12.7). Carcass dissection was performed using the method described by Walstra and Merkus (1995), and standard carcass traits were measured. The following performance traits were also recorded: live weight at slaughter, feed conversion ratio, and average daily gain; back fat depth (see Dvořáková et al., 2012); intramuscular fat in the neck, shoulder, and *m.l.l.t.*, and fat content of the belly (determined by the Soxhlet method, which uses gravimetric determination in accordance with the Czech Standard, ČSN ISO 1443 (1973); for information on the belly see Stupka et al., 2004); fat and muscle depth and lean meat percentage (measured with Fat-O-Meter (SFK, Viborg, Denmark) (Šprysl et al., 2007) and TP – two-point method (Krška et al., 2002)); carcass yield; weight of carcass body; pH in *m.l.l.t.* (45 min and 24 h *post mortem*) and temperature in *m.l.l.t.* (45 min *post mortem*). The muscle depth and area of *m.l.l.t.* were measured by the LUCIA (Laboratory Imaging) program.

For practical and organizational reasons, individual performance traits were not recorded for all of the animals; therefore, different numbers of pigs were analyzed per trait.

Table 2. Information on PCR primers and conditions used for resequencing of porcine *SORT1*, *EMP3*, *IL18*, and *BTG2*

Source sequence	Primer name	Primer sequence (5'- 3')	Amplicon size (bp)	T_a (°C)	$MgCl_2$ (mM)	Poly-merase	Location
ENSSSCG 00000006831	SORT1-A	CCATGCCCACTCCATTCTAAAT	1177	55	1.5	LA	intron 3
	SORT1-B	GGCCACAGGAAAGGAACAATAA					intron 5
	SORT1-C	TGCTACCAGCCTTTGTAGAACT	1534	55	1.5	LA	intron 12
	SORT1-D	CAGCCAGAAGAAATTAAACTGAT					intron 14
ENSSSCG 00000023377	EMP3-Q	CCGCCAGCATTCAAATGACT	1799	59	1.5	LA	5' upstream
	EMP3-R	GTCCCGTCCGCACTGTCTATG					exon 1; 5' UTR
	EMP3-I	GGTGGGGGCCCGGAGAATGAG	1555	65	0.9	LA	exon 1; 5' UTR
	EMP3-J	GGGCGTGGCGGCTGGAGTCTG					intron 2
	EMP3-A	CTCCTCCTGCTGGTGGTCTCT	421	55	1.0	LA	exon 2
	EMP3-B	GCCCACGTTTTGTTGTCACT					exon 3
	EMP3-E	AAACGTGGGCCTGCAGTAATG	1158	55	1.0	LA	exon 3
	EMP3-F	CTCGCCGCATGGTGTACA					exon 4
	EMP3-C	TGCGGCGAGGAGGGCTCTTCT	1049	57	1.0	LA	exon 4
	EMP3-D	GGGCGAGCGGGAAGGCTACC					exon 5
ENSSSCG 00000015037	IL18-Ex2_4A	TGCTGGACTTCTGAAATTAGAG	1087	62	1.5	*Taq*	intron 1
	IL18-Ex2_4B	GGGATGGCTTTTGTCTACAG					intron 2
	IL18-Ex4_5A	TCAGACTGCACAAAAAGGAAGG	963	62	1.5	*Taq*	intron 3
	IL18-Ex4_5B	AGTAGCATGACCCCTAGCACTGT					intron 4
	IL18-Ex5_8A	ATTACCGTGAGCTGCAGTATAG	1009	62	1.5	*Taq*	intron 4
	IL18-Ex5_8B	AAGGTTGTAGCCAAAACTTAGA					intron 5
ENSSSCG 00000028322	BTG2-E	CCAGCCAACTGCACTTGTGAC	1138	57	1.0	LA	5' upstream
	BTG2-F	GAGGCTGGAGAGGAAACCTACA					exon 1
	BTG2-A	GGCACCTCTCGGGTAAAGT	1244	59	1.5	LA	5' upstream
	BTG2-B	AAGGCCTCTGGAGACCTATGAG					intron 1
	BTG2-C	CTGGCTGGGGAGAGACCTATCT	1172	59	1.5	LA	intron 1
	BTG2-D	CTCAAGCGCACAACGATTCT					exon 2

LA = polymerases mix (Top-Bio, Prague, Czech Republic), *Taq* = Taq DNA Unis polymerase (Top-Bio), T_a = annealing temperature

All pigs were slaughtered according to the protocols for certified Czech slaughterhouses under the supervision of an independent veterinarian.

Resequencing and polymorphism analysis. Genomic fragments of *SORT1*, *EMP3*, *IL18*, and *BTG2* genes from DNA of Wild Boar, Large White, and Meishan pigs were amplified by PCR with the use of primers designed on the basis of the sequences of the pig genes available in the Pig Ensembl database (http://www.ensembl.org/; Sscrofa10.2). The primers used for the amplification of the fragments and for sequencing are shown in Table 2. Sequencing was performed using an ABI PRISM®3130 Genetic Analyzer (Applied Biosystems, Foster City, USA) and the sequences were deposited in the EMBL/GenBank database. Through comparison of the sequences polymorphisms were identified (Table 3). Selected polymorphisms were analyzed by PCR-RFLP using the primers and conditions that are presented in Table 4. *RYR1* genotyping (Table 4) was accomplished according to Brem and Brenig (1992). The polymorphisms were genotyped in purebred pigs (estimation of allele frequencies), in the Hohenheim Meishan × Pietrain F_2 pigs (linkage mapping), and in commercial crosses (association analysis with performance traits).

Linkage mapping. Linkage mapping of the four studied genes was performed on the Hohenheim three-generation pedigree of Meishan × Pietrain using the two-point and multi-point analyses of the CRI-MAP package, Version 2.4 (Green et al., 1990).

Table 3. SNPs and indels identified in porcine *SORT1*, *EMP3*, *IL18,* and *BTG2* genes by comparative sequencing

Gene	Accession No.[1]	Location	SNP/indel	Note
SORT1	HE650898	exon 4	g.82T>C	silent
		intron 4	g.191A>G	
		intron 5	g.1024T>G	
	HE650902	intron 12	g.133C>T	
		intron 13	g393delG, g.512T>C, g.557C>G, g.577C>G, g.580A>G, g.684C>T, g.699A>G, g.732C>T, g.796C>T, g.813C>G, g.840A>G, g.874G>C, g.888C>A, g.1092T>G, g.1126G>C	
		exon 14	g.1327G>C	silent
		intron 14	g.1399T>A	
EMP3	HE686968	intron 1	1744T>G	
		intron 3	g.3923delC	
		intron 4	g.4359C>A, g.4376C>A, g.4566delG, g.4717A>T	
IL18	HE681921	intron 1	g.30T>C	
		exon 2	g.67A>G	silent
		intron 2	g.233A>T, g.324T>A, g.344T>C, g.368C>G, g.429G>A, g.447C>T, g.486_490delATTTT, g.608G>A, g.619A>G, g.642A>G, g.649C>T, g.702A>G, g.756A>T, g.768C>T, g.852A>G, g.952G>A, g.960C>T	
	HE681923	intron 3	g.4G>A, g.47_58delGGTCCAAAAGTG, g.107G>A, g.141A>G, g.205A>G, g.256C>T, g.258G>A, g.274G>A, g.311G>A, g.330_331insAAAAA, g.361C>T	
		intron 4	g.705C>T, g.808T>C, g.824G>A	
	HE681924	intron 4	g.11G>T, g.226G>A, g.243_244delAA, g.272G>C	
		intron 5	g.705G>A, g.728C>T, g.784G>C, g.867A>G	
BTG2	FR751404	5' UTR	g.952T>G	
		intron 1	g.1193T>C, g.1224G>T, g.1243G>A, g.2094G>C, g2264C>T	
		3' UTR	g.2905_2906insT, g.2930_2931delAA, 2947T>A	

[1]to define the positions of SNPs, a single sequence is referred to for each of the sequenced fragments

Association analysis. Associations between genotypes and growth and carcass traits were performed separately for the genes *SORT1*, *EMP3*, *IL18*, and *BTG2* in the commercial Czech populations by means of the GLM procedure (type IV) of SAS software, Version 9.1.3. (SAS Institute Inc., Cary, USA, 2004). The statistical model was:

$$Y_{ijklmn} = \mu + genotype_i + RYR_j^{*} + cross_k^{**} + sex_l + diet_m^{***} + b(s\_age_{ijklmn} - S\_age) + e_{ijklmn}$$

where:

Y_{ijklmn} = trait value of animal *n*

μ = estimated mean value of a trait for the animals included in the model

$genotype_i$ = effect of the genotype class for one of the studied genes (*SORT1*, *EMP3*, *IL18,* or *BTG2*) (*i* = 1–3)

RYR_j = effect of the *RYR1* genotype – only in *EMP3* analysis (*j* = 1, 2)

$cross_k$ = effect of the crossbred combination (*k* = 1–10)

sex_l = effect of sex (*l* = 1, 2)

$diet_m$ = effect of diet (*m* = 1, 2)

b = linear regression value

s_age_{ijklmn} = age at slaughter of animal *n*

S_age = estimated average age at slaughter

e_{ijklmn} = random residual effect

*the separately performed association analysis for *EMP3* in pigs of *RYR1 CC* homozygotes did not include the effect of the *RYR1* gene

**the effect of crossbred populations was not included in the separate association analyses of the cross PN × (CZL × CZLW)

***thirty-five pigs of the PN × (CZL × CZLW) cross were fed with a restriction diet (37.4 MJ ME/day) from live weight of 90 kg to slaughter weight

Table 4. Information on primers, PCR, and RFLP conditions for SNP analysis of *SORT1*, *EMP3*, *IL18*, *BTG2*, and *RYR1*

Gene	Source sequence	Primer sequences (5'-3')	Amplicon (bp)	$MgCl_2$ (mM)	Polymerase	T_a (°C)	SNP type	Restriction enzyme	PCR-RFLP pattern (bp)	Location of SNP
SORT1	ENSSSCG 00000006831	TGCTACCAGCCTTTGTAGAACT CCCAAAGGAAAGAGAGGATAGT	1041	1.0	LA	57	HE650901:g.796C>T	*Hpa*II	*T*: 711 + 330 *C*: 533 + 330 + 178	intron 13
EMP3	FP565349 NC_010448.2	TGCGGCGAGGAGGGCTCTTCT GGGCGAGCGGGAAGGCTACC	1049	1.0	LA	57	HE686968:g.4376C>A	*Xap*I	*C*: 1049 *A*: 889 + 160	intron 4
IL18	ENSSSCG 00000015037	TGCTGGACTTCTGAAATTAGAG CCTGTATTTGGTCTAATGTTTTG	217	1.0	*Taq*	55	HE681921:g.67A>G	*Msp*I	*A*: 217 *G*: 124 + 93	exon 2, (silent)
BTG2	ENSSSCG 00000015269	TTCCCCCTCCTCTCGAAATAGT CGATCTCCGGGAGCATATCT	213	1.5	LA	55	FR751404:g.952T>G	*Cac*8I	*T*: 173 + 40 *G*: 94 + 79 + 40	5' UTR
RYR1	X65504	GTGCTGGATGTCCTGTGTTCCCT CTGGTGACATAGTTGATGAGGTTTG	134	1.0	*Taq*	69	X65504:g.192C>T	*Hha*I	*T*: 134 *C*: 84 + 50	exon 17

LA = polymerases mix (Top-Bio, Prague, Czech Republic), *Taq* = Taq DNA Unis polymerase (Top-Bio)

The estimates of the genotype classes were assessed by *t*-test. In cases of significant SNP effects, the Bonferroni test was performed using the MULTTEST procedure of SAS.

RESULTS AND DISCUSSION

Resequencing, polymorphism detection, allele frequencies, and linkage mapping

SORT1. PCR fragments from two regions of the gene including exons 4–5 and 13–14, respectively, were comparatively sequenced (CZLW, Wild Boar, Meishan) (Table 2) and the sequences were deposited in the EMBL/GenBank database (Accession numbers HE650897–HE650904). Altogether, 21 polymorphisms were detected (20 SNPs and one indel). They are listed in Table 3 and can also be retrieved from the sequences deposited in the EMBL/GenBank database. Two SNPs were in exons (silent); all other polymorphisms were in introns. SNP HE650901:g.796C>T (intron 13) was used for genotyping.

EMP3. PCR fragments containing more than 5000 bp of the genomic sequence of porcine *EMP3* (5' upstream region, complete exons 1–4, intervening introns, and incomplete exon 5) were comparatively sequenced (CZLW, Wild Boar, Meishan) (Table 2) and the sequences were deposited in the GenBank/EMBL database (Accession numbers HE686968, HE686969, and HG324061). Only six polymorphisms were observed (four SNPs and two indels) (Table 3), all in introns. SNP HE686968:g.4376C>A was used for genotyping.

IL18. PCR fragments harbouring exons 2, 4, and 5, respectively, and flanking sequences of introns were comparatively sequenced (CZLW, Wild Boar, Meishan) (Table 2) and the sequences were deposited in the GenBank/EMBL database (Accession numbers HE681920–HE681925, HG416932–HG416934). In total, 41 polymorphisms were found (37 SNPs and 4 indels) (Table 3). The polymorphic sites can also be retrieved from the GenBank sequences. From the detected polymorphisms, 40 were in introns and one in an exon. SNP HE681921:g.67A>G, which is located in exon 2 (silent mutation), was used for genotyping.

BTG2. Through comparative sequencing (CZLW, Wild Boar, Meishan) (Table 2) the genomic sequence of the whole coding region of the *BTG2* gene was obtained (GenBank/EMBL database, FR751404,

Table 5. Frequencies of alleles in *SORT1*, *EMP3*, *IL18,* and *BTG2* genes in eight pig breeds and Wild Boar

Breed	*SORT1*			*EMP3*			*IL18*			*BTG2*		
	SNP HE650901: g.796C>T (*Hpa*II)			SNP HE686968: g.4376C>A (*Xap*I)			SNP HE681921: g.67A>G (*Msp*I)			SNP FR751404: g.952T>G (*Cac*8I)		
	n	*T*	*C*	*n*	*C*	*A*	*n*	*A*	*G*	*n*	*T*	*G*
Czech Large White	37	0.45	0.55	19	0.74	0.26	383	0.36	0.64	17	0.35	0.65
Czech Landrace	31	0.77	0.23	28	0.75	0.25	32	0.69	0.31	23	0.04	0.96
Czech Meat Pig	15	0.50	0.50	15	0.53	0.47	15	0.57	0.43	15	0.17	0.83
Pietrain	21	0.36	0.64	21	0.62	0.38	21	0.62	0.38	21	0.07	0.93
Black Pied Prestice	17	0.76	0.24	7	0.71	0.29	17	0.47	0.53	7	0.14	0.86
Hampshire	9	0.44	0.56	5	0.90	0.10	9	0.83	0.17	7	0.79	0.21
Duroc	34	0.03	0.97	23	0.87	0.13	30	0.37	0.63	10	0.25	0.75
Meishan	19	1.00	0.00	18	0.25	0.75	18	0.42	0.58	12	0.05	0.95
Wild Boar	3	0.67	0.33	3	1.00	0.00	3	0.50	0.50	3	1.00	0.00

HG324058–HG324060). Nine polymorphisms were detected (seven SNPs and two indels) (Table 3). Of these, four polymorphisms were in 5' and 3' UTR, and five SNPs were in intron 1. SNP FR751404:g.952T>G (5' UTR) was used for genotyping.

The frequencies of alleles of the tested SNPs of the four genes in eight breeds of pigs and Wild Boar are presented in Table 5.

By linkage analysis in the Hohenheim Meishan × Pietrain cross, the genes were mapped to previously constructed linkage maps. The maps show the positions of the studied genes relative to other genes and markers on the respective chromosomes. Sex averaged maps with distances in Kosambi cM are presented:

SORT1 was mapped to the SSC4 linkage map originally described by Cepica et al. (2003b): *SW489* – 21.7 – *MYC* – 4.2 – *SW835* – 16.3 – *SWR73* – 4.6 – *S0135* – 10.2 – *SW1073* – 6.2 – *SW1089* – 2.7 – *V-ATPase* – 3.0 – *ATP1B1* – 5.3 – *LMX1A* – 0.0 – *S0073* – 4.4 – *ATF6* – 1.9 – *HSD17B7* – 0.2 – *SDHC* – 0.0 – *MPZ* – 0.2 – *APOA2* – 1.1 – *CASQ1* – 1.1 – *ATP1A2* – 0.4 – *MEF2D* – 2.5 – *LMNA* – 0.6 – *GBA* – 0.5 – *PKLR* – 1.5 – *IVL* – 8.1 – *EAL* – 1.9 – *ATP1A1* – 0.1 – *NGFB* – 2.6 – *AMPD1* – 2.5 – *TSHB* – 8.9 – *SW2435* – 4.3 – ***SORT1*** – 9.6 – *AGL* – 12.6 – *S0097* – 3.0 – *ABCD3* – 0.0 – *CNN3*.

EMP3 was mapped to the SSC6 linkage map originally constructed by Yue et al. (2003): *S0035* – 24.5 – *SW1329* – 33.7 – *SW1057* – 16.3 – *FTO* – 6.5 – *S0087* – 15.7 – *RYR1* – 1.9 – *LIPE* – 0.9 – *TGFB1* – 1.2 – *A1BG* – 1.4 – *EAH* – 0.0 – ***EMP3*** – 3.3 – *SKI* – 15.4 – *FABP3* – 3.9 – *ID3* – 14.3 – *S0146* – 9.8 – *S0003* – 16.3 – *SW824* – 50.1 – *P3* – 20.0 – *EAO*.

IL18 and ***BTG2*** were mapped to the SSC9 map originally prepared by Cepica et al. (2003a): *EAK* – 19.0 – *HPX* – 6.5 – *SW21* – 26.5 – *SW911* – 15.5 – *SLN* – 1.4 – ***IL18*** – 8.7 – *SW2074* – 11.6 – *APOA1* – 15.1 – ***BTG2*** – 8.5 – *LPR* – 4.0 – *EAN* – 11.3 – *NAMPT* – 5.5 – *MYOG* – 2.2 – *SW1435* – 2.4 – *SW2093* – 10.3 – *GLUL* – 10.8 – *S0114* – 22.2 – *EAE* – 7.9 – *SW1349*.

Association analysis

Association analyses were performed separately for the Pietrain × (Czech Landrace × Czech Large White) population, and the combined pig populations (joint population (Table 1). Although the combined population is less suitable for association analysis, here it is included to show that for some traits the same associations were observed as in the PN × (CZL × CZLW) population. The polymorphisms in *SORT1*, *EMP3*, *IL18,* and *BTG2* and their associations with growth rate, carcass traits, muscling, fatness, and meat quality traits were studied. The results (significant at $P < 0.05$) are shown as representative traits in Table 6; some highly correlated traits are not shown. In Table 6, the traits significant at $P < 0.05$ after Bonferroni correction are also indicated.

SORT1, SNP HE650901:g.796C>T. In the commercial crosses, we found several significant associations between *SORT1* genotypes and carcass traits (Table 6). Of greater interest are the traits lean meat percentage (FOM) and depth of *m.l.l.t.* in the PN × (CZL × CZLW) population, which had significantly higher values in genotype *CC* (and

Table 6. Results of association analysis between *SORT1*, *EMP3*, *IL18,* and *BTG2* genotypes and carcass traits in the commercial pig populations

Gene	Trait	Population[1]	*n*	Genotype, LSM ± SE (*n*)[2]			*P*
SORT1				*TT*	*TC*	*CC*	
	carcass weight 45 min *p.m.* (kg)	PN × (CZL × CZLW)	156	89.01 ± 2.28^{a} (18)	94.43 ± 1.28^{b} (73)	92.53 ± 1.16^{ab} (65)	0.030*
	lean meat (FOM) (%)	PN × (CZL × CZLW)	156	55.71 ± 0.67^{a} (18)	57.20 ± 0.38^{b} (73)	57.19 ± 0.34^{b} (65)	0.043
	intramuscular fat content in shoulder (%)	PN × (CZL × CZLW)	81	2.66 ± 0.20^{a} (9)	2.13 ± 0.12^{b} (36)	2.18 ± 0.10^{b} (36)	0.026*
	belly weight relative to carcass weight (%)	PN × (CZL × CZLW)	85	17.26 ± 0.37^{a} (9)	16.39 ± 0.20^{b} (40)	16.65 ± 0.18^{ab} (36)	0.036
	depth of *m.l.l.t.* (mm)	PN × (CZL × CZLW)	85	70.37 ± 1.92^{a} (9)	72.41 ± 1.03^{ab} (41)	74.94 ± 0.95^{b} (35)	0.032*
	lean meat (FOM) (%)	joint	610	52.83 ± 0.47^{a} (132)	53.83 ± 0.37^{b} (280)	53.24 ± 0.41^{ab} (198)	0.025
	intramuscular fat in *m.l.l.t.* (%)	joint	317	2.00 ± 0.12^{ab} (70)	1.93 ± 0.10^{a} (146)	2.13 ± 0.10^{b} (101)	0.042
EMP3				*CC*	*CA*	*AA*	
	carcass weight 45 min *p.m.* (kg)	PN × (CZL × CZLW)	152	91.02 ± 1.79^{ab} (38)	92.51 ± 1.12^{a} (96)	86.42 ± 2.53^{b} (18)	0.016*
	lean meat (TP) (%)	PN × (CZL × CZLW)	153	61.00 ± 0.55^{a} (38)	60.78 ± 0.34^{a} (97)	62.64 ± 0.78^{b} (18)	0.017
	main meat parts including fat relative to carcass weight (%)	PN × (CZL × CZLW)	87	68.87 ± 0.41^{a} (21)	69.98 ± 0.27^{b} (54)	69.84 ± 0.58^{ab} (12)	0.014*
	fat depth on *m. gluteus medius* (TP) (mm)	PN × (CZL × CZLW)	153	12.59 ± 0.80^{a} (38)	12.53 ± 0.50^{a} (97)	9.65 ± 1.12^{b} (18)	0.010*
	pH 45 min *p.m.* in *m.l.l.t.*	PN × (CZL × CZLW)	119	6.38 ± 0.06^{ab} (28)	6.43 ± 0.04^{a} (78)	6.24 ± 0.09^{b} (13)	0.028*
	temperature 45 min *p.m.* in *m.l.l.t.* (°C)	PN × (CZL × CZLW)	122	34.67 ± 0.48^{a} (29)	35.78 ± 0.32^{b} (79)	36.65 ± 0.74^{b} (14)	0.014
	carcass weight 45 min *p.m.* (kg)	joint	747	92.80 ± 0.90^{ac} (387)	93.16 ± 0.88^{c} (318)	89.84 ± 1.64^{b} (42)	0.021
	main meat parts (kg)	joint	521	29.41 ± 0.38^{A} (293)	29.58 ± 0.37$^{\alpha}$ (205)	27.52 ± 0.73$^{B\beta}$ (23)	0.001*
	fat depth (FOM) (mm)	joint	607	17.52 ± 0.40^{a} (318)	17.23 ± 0.41^{ab} (253)	16.09 ± 0.76^{b} (36)	0.038
	pH 45 min *p.m.* in *m.l.l.t.*	joint	643	6.34 ± 0.03^{A} (349)	6.34 ± 0.03^{A} (259)	6.19 ± 0.06^{B} (35)	0.005*
IL18				*AA*	*AG*	*GG*	
	ham weight relative to carcass weight (%)	PN × (CZL × CZLW)	83	30.06 ± 0.40^{a} (16)	29.19 ± 0.25^{ab} (46)	28.84 ± 0.36^{b} (21)	0.019*
	intramuscular fat in ham (%)	PN × (CZL × CZLW)	67	2.49 ± 0.36^{a} (14)	3.13 ± 0.23^{ab} (35)	3.58 ± 0.30^{b} (18)	0.015
	depth of *m.l.l.t.* (FOM) (mm)	PN × (CZL × CZLW)	152	67.20 ± 1.29^{ab} (30)	67.19 ± 0.81^{a} (80)	64.14 ± 1.10^{b} (42)	0.018
	depth of *m. gluteus medius* (TP) (mm)	PN × (CZL × CZLW)	151	83.13 ± 1.38^{a} (30)	81.28 ± 0.86^{ab} (80)	79.00 ± 1.18^{b} (41)	0.017*
	temperature 45 min *p.m.* in *m.l.l.t.* (°C)	PN × (CZL × CZLW)	121	35.40 ± 0.48^{ab} (25)	35.73 ± 0.29^{a} (66)	34.59 ± 0.43^{b} (30)	0.027*
	lean meat (FOM) (%)	joint	603	52.81 ± 0.46^{a} (130)	53.38 ± 0.36^{ab} (319)	53.97 ± 0.46^{b} (154)	0.027*
	loin weight (including fat) relative to carcass weight (%)	joint	507	17.73 ± 0.16^{ac} (117)	17.80 ± 0.15^{c} (261)	18.09 ± 0.18^{b} (129)	0.027*
BTG2				*TT*	*TG*	*GG*	
	intramuscular fat content in *m.l.l.t.* (%)	PN × (CZL × CZLW)	75	[3]	1.29 ± 0.10^{a} (26)	1.53 ± 0.07^{b} (46)	0.046
	drip loss in *m.l.l.t.* (%)	PN × (CZL × CZLW)	70	[3]	6.71 ± 0.75^{a} (19)	8.77 ± 0.46^{b} (48)	0.013
	intramuscular fat content in shoulder (%)	joint	212	2.35 ± 0.20^{AB} (14)	2.20 ± 0.11^{A} (57)	2.49 ± 0.08^{B} (141)	0.008*
	fat in the belly 1 (%)	joint	311	24.77 ± 1.79^{a} (18)	27.71 ± 1.02^{ab} (105)	28.40 ± 0.86^{b} (188)	0.031
	drip loss in *m.l.l.t.* (%)	joint	264	10.41 ± 0.67^{a} (21)	8.99 ± 0.36^{b} (91)	9.48 ± 0.29^{ab} (152)	0.046*

p.m. = *post mortem*, *m.l.l.t.* = *m. longissimus lumborum et thoracis*, FOM = measured with Fat-O-Meter (Šprysl et al., 2007), TP = two-point measurement method, PN = Pietrain, CZL = Czech Landrace, CZLW = Czech Large White

[1]see Table 1

[2]values with different superscripts in the row differ significantly: $^{a-c}P < 0.05$, $^{A,B}P < 0.01$, $^{\alpha,\beta}P \leq 0.001$

[3]as only three pigs of genotype *TT* were observed, they were excluded from the analysis

*after Bonferroni correction, $P < 0.05$

TC) compared with *TT*. In the joint population, lean meat showed the same trend. Intramuscular fat in *m.l.l.t.* was lower in *TC* heterozygotes.

In a Wild Boar × Pietrain F_2 cross, significant QTL for ham meat weight, dressing B and shoulder meat weight were found in this chromosome interval of SSC4 (Cepica et al., 2003b). No significant QTL were detected in this chromosome interval in Meishan × Pietrain and Wild Boar × Meishan crosses (Cepica et al., 2003b; Geldermann et al., 2010). In a Berkshire × Yorkshire F_2 cross, the position of *SORT1* is expected to be in the chromosome interval for QTL for loin eye area, carcass weight, last rib back fat, and average daily gain (Malek et al., 2001).

EMP3, SNP HE686968:g.4376C>A. In commercial populations, the analysis was first performed with co-factor RYR1 included in the model. Significant associations ($P < 0.05$) of *EMP3* genotypes with several traits (mainly growth and muscling, and also pH 45 min *post mortem*) were found (Table 6). In the PN × (CZL × CZLW) crossbred, heterozygotes *CA* were superior for some growth and muscling traits. Similar results were obtained when the association analysis of *EMP3* was performed only for pigs with genotype *RYR1 CC* (data not shown; the frequency of allele *RYR1 T* was 0.12, and only heterozygotes *CT* were observed). In the joint population, similar associations were observed (Table 6).

Interestingly, the numbers of *EMP3* genotypes significantly deviated from the Hardy-Weinberg equilibrium (HWE) both in the PN × (CZL × CZLW) crossbred and the joint population ($P < 0.001$ and $P < 0.05$, respectively; excess of heterozygotes *CA* and deficit of both homozygotes), while the other three polymorphisms (*SORT1*, *IL18,* and *BTG2*) were in HWE in both studied populations.

On the basis of linkage mapping in Meishan × Pietrain F_2 pedigree pigs, it is obvious that *EMP3* is located distally from *RYR1*, in the QTL interval for numerous carcass and meat quality traits, and the same situation also appears to be in the Wild Boar × Pietrain F_2 cross (Yue et al., 2003). Numerous QTL were detected distally, close to *RYR1* (see http://www.animalgenome.org/cgi-bin/QTLdb/SS/index). For example, Mohrmann et al. (2006) detected QTL for several fatness and muscling traits in this region in an F_2 cross of two commercial pig lines. They concluded that additional loci closely linked to the *RYR1* gene are involved in phenotypic variation in those traits. *EMP3* could be one of them. Edwards et al. (2008), in an F_2 Duroc × Pietrain resource population (with the *RYR1 CC* genotype), found QTL for several fatness and muscling traits distally from marker *S0220*, which is close to *RYR1* (Archibald et al., 1995). As growth and several carcass traits are associated with *EMP3* polymorphism, this gene deserves to be studied in more detail.

IL18, SNP HE681921:g.67A>G. Significant associations of *IL18* in the commercial crosses are summarized in Table 6. In the PN × (CZL × CZLW) population, genotypes *AA* and *AG* were associated mainly with higher values for some meat traits (depth of *m.l.l.t.* and *m. gluteus medius*, percentage of ham weight relative to carcass weight), while genotype *GG* was associated with a higher value for intramuscular fat in ham. In the joint population, significant associations were observed for two traits (Table 6).

IL18 is located on SSC9, approximately 9 cM proximal to marker *SW2074*. In this chromosome interval in a Duroc × Landrace F_2 population, Rohrer et al. (2006) detected QTL for total lean content, fat percentage, and loin eye area. In the same region, Wada et al. (2000) identified QTL for average daily gain (4–13 weeks) in a Göttingen minipig × Meishan F_2 population. These results, as well as ours, indicate that this chromosomal region harbours genes that play roles in growth and meat quality. One of the candidates is *IL18*.

BTG2, SNP FR751404:g.952T>G. The gene is localized on SSC9, similarly as *IL18*, but the distance between the two genes is 35.4 cM, so they segregate nearly independently. In the commercial cross PN × (CZL × CZLW) only three animals were of genotype *TT*, so this class was excluded from the association analysis. Therefore, only *TG* and *GG* genotypes could be compared. Except for a couple of associations pertaining to meat quality traits, no associations with muscling traits were found. Intramuscular fat content was higher in the *GG* genotype, and drip loss in *m.l.l.t.* was lower in the *TG* genotype. In the joint population similar associations were observed (Table 6).

It is of interest that gene *APOA1* (apolipoprotein A1) and locus *LPR* (lipoprotein Lpr) are located not far from *BTG2*. In the homologous segment of human chromosome 11q23.3, there is a cluster of several apolipoprotein genes (*APOA1*, *APOA4*, *APOA5,* and *APOC3)* (http://www.ensembl.org/), of which some (*APOA1*, *APOA4*) are involved in fat digestion and absorption.

Mo et al. (2011) performed association analysis of an intronic *BTG2* polymorphism with carcass traits in a Large White × Meishan F_2 resource population and found associations with buttock fat thickness, fat percentage, lean meat percentage, ratio of lean to fat, and carcass length. In an F_2 population created by crossing Pietrain, Large White, Landrace, and Leicoma, Duthie et al. (2008) identified several QTL at a position around 90 cM on SSC9, where *BTG2* may be located. These were average daily gain (120–140 kg), neck weight without external fat, external shoulder fat weight, protein accretion rate (120–140 kg), and lipid accretion rate (120–140 kg). Paszek et al. (2001), in a Meishan × Yorkshire F_2 family, detected QTL for carcass length in this chromosomal interval. Minor QTL for growth and fattening traits were identified in this region in a Wild Boar × Pietrain F_2 family (Cepica et al., 2003a). *BTG2*, due to its biological functions (Mo et al., 2011), may be a candidate for some growth and carcass traits.

All polymorphisms identified in the four genes are most likely non-functional mutations. Most of them are in introns, and those which are in exons are silent mutations or located in 5' UTR or 3' UTR. Out of the four tested polymorphisms two were in introns, one in an exon (silent), and one in 5' UTR. The observed associations are most probably due to linkage disequilibrium between the studied genotypes and performance traits. Further studies will be needed to search for causative mutations that could influence the traits of interest.

In conclusion, through comparative sequencing, we revealed numerous polymorphisms in porcine candidate genes expressed in skeletal muscles, *SORT1*, *EMP3*, *IL18,* and *BTG2*. None of the polymorphisms was a missense mutation. Linkage analysis in a Meishan × Pietrain F_2 pedigree has shown the positions of the studied genes relative to other genes and markers on the respective chromosomes. Selected SNPs were associated with several carcass traits in porcine commercial crosses. Our results indicate that the polymorphisms tested are most probably markers that are in linkage disequilibrium with unknown causative mutations for the studied performance traits. SNPs studied in these analyses could be potentially useful in marker assisted selection and in increasing pork production efficiency. However, further research on other populations is needed to confirm these results and to identify causative mutations.

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