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Database of SNPs in candidate genes potentially associated with yield and quality of pork*

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A growing number of mutations within genes potentially associated with pork production and quality are neither classified nor described in a way facilitating the design and interpretation of experiments with the use of high throughput screening techniques. The aim of the paper presented here was to process and catalogue the publicly available information on single nucleotide polymorphisms (SNPs) located within genes that are directly, indirectly or potentially associated with pig muscle growth and metabolism affecting yield and quality of pork.
A constructed database is presented containing 153 SNPs within 113 genes. Among the 153 SNPs, 152 were found to be single nucleotide substitutions (117 transitions and 35 transversions). Additionally, one indel was included. All collected SNPs are described in a way enabling the automatic downloading of GenBank records to specialized software and simultaneous designing of PCR primers and allele-specific probes to be used in microarray technology.
It is believed that collection of SNPs presented in this paper will serve as a reliable resource for SNP interaction studies, clarifying the genetic determination of pig muscle growth and metabolism, and – after validation in different breeds – also for parentage control, traceability tests and evolutionary studies in pig breeding.

KEY WORDS: candidate genes / database / growth / pig / pork / SNP

Genetic determinants of pig muscle growth and metabolism are poorly understood. So far, only a few genetic markers associated with pork production traits have been identified: *RYR1* (ryanodine receptor) gene [Fujii *et al.* 1991] affecting meat quality

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(PSE meat), RN mutation ("Napole" technological yield) [Milan *et al.* 2000] and other mutations in the protein kinase AMP-activated gamma-3 subunit gene affecting meat quality [Ciobanu *et al.* 2001], insulin-like growth factor 2 gene, associated with muscle growth [Van Laere *et al.* 2003] and melanocortin-4 receptor gene probably influencing fatness, growth and feed intake in pigs [Kim *et al.* 2000].

As muscle growth and consequently pork yield and quality are certainly polygenic traits determined by hundreds of genes, probably many of SNPs remain undiscovered. Such mutations may be detected through a fine mapping, physical mapping and comparative sequencing strategy or by typing candidate gene polymorphisms. "Candidate" status of a gene means that it is involved in biochemical and physiological processes characteristic for the tissue or trait of interest. Such mutations can not only be located in coding sequences and sometimes substitute amino acids, but also in regulatory elements of the gene, thus affecting gene transcription.

It is thought that the simultaneous genotyping of many informative SNPs will lead to a better understanding of the genetic background of pork traits. The functional effects of polymorphisms are mostly evaluated for single SNPs. In near future, gene interaction studies should give better insight into the genetic basis of different traits, mostly involving production and quality of pork. Interaction studies, including many SNPs require a database of SNPs arranged and described in a way which facilitate the design and interpretation of results of experiments with the use of high throughput screening techniques. Currently the best method for typing SNPs determining complex traits is DNA microarray [reviewed by Syvänen 2001, and Kamiński 2002]. This technology, however, requires precise DNA sequence information, particularly upon SNPs type and location.

The general aim of this work was to construct a database of publicly available polymorphic sequences that are directly, indirectly or potentially associated with pig muscle growth, yield and metabolism.

SNP definition, database structure

SNPs (single nucleotide polymorphisms) are single base pair positions in genomic DNA at which different sequence alternatives (alleles) exist in normal individuals in some population(s), wherein the least frequent allele has an abundance of 1% or greater [Brookes 1999]. In practice, the term SNP is typically used more loosely and encompasses many different types of subtle sequence variations (including small deletions and insertions) with the frequency of a rare allele being less than 1%. To maintain the clarity of this paper, the latter SNP definition has been employed.

All records of the database were arranged in a table (Tab. 1) consisting of the following columns: *Sus scrofa* chromosome, *locus* symbol, gene name (definition), SNP position (within GenBank sequence), significance, type and position of mutation in the gene stucture, (e.g. exon, intron, 5'flanking, 5'UTR, *etc*), restriction site potentially useful in PCR-RFLP analysis, GenBank accession number, and reference.

The primary source of records was the GenBank database (NCBI, www.ncbi.nlm.nih.gov) in which 1559 records (genes or nucleotide sequences) were found by searching for "Sus scrofa and variation". The following additional resources were also used: (i) the world-wide bibliographic databases (Medline, CAB, BIOSIS), (ii) pig mapping genome databases (NAGRP Pig Genome Coordination Program http://www.animalgenome.org/pigs/ and (iii) ARKdb http://iowa.thearkdb.org). Column "Reference" contains references mostly to the documented functional effects of SNP as well as allele frequency data. All of these resources were first previewed and evaluated to ensure they contained at least three elements: GenBank acc. no, position of SNP and minimum length of sequence 25-30 bp of DNA from both flanking sides of the SNP. For many records, individual analysis was necessary to establish position of the SNP which differed between GenBank and respective reference. In some cases, SNPs marked in the GenBank sequence were translated at the protein level or annotated by additional information gained from papers.

Database content and its potential applications

A database was constructed containing 153 SNPs within 113 genes (Tab. 1). Among the 153 SNPs, 152 were single nucleotide substitutions, (117 transitions and 35 transversions). Additionally, one indel was included. Most SNPs were found to be located in the coding regions of the genome (64 missense mutations) and could had direct impact on the phenotype of an individual. Moreover 25 SNPs in 5' flanking regions (promoters) and UTRs were included. These SNPs may affect the yield of a gene expression. All presented SNPs can also be used as markers for unknown adjacent genomic regions.

The most important feature of the SNP database is the precise information on the nature and location of a given SNP. Each SNP is described in the same way, for example, the first SNP in Table 1 (472 Y) means that in the sequence recorded under GenBank acc. no. AF034974, at position 472, mutation can occur with two alleles: T or C. This uniform method of SNP description enables the automatic downloading of GenBank records to specialized software and simultaneous design of PCR primers and allele specific probes used in microarray technology.

Ordering different data in the same way revealed that some papers or GenBank records contain insufficient, conflicting, or even error-prone sequence information. SNPs were also supplemented with some important information on their function or significance. Most of these annotations indicate the type of mutation: missense, silent or frame shift (if missense – an amino acid substitution is described) and a SNP location in the gene structure: intron, exon, 5'flanking, promoter or UTR. For several SNPs no information is available in which part of the gene they are located. Some SNPs were located within putative (computational) or experimentally confirmed binding sites of transcription factors.

Table 1. SNPs database content. Abbreviations according to IUPAC code: M, A/C; R, A/G; W, A/T; S, C/G; Y, C/T; K, G/T

Chro-mosome	*Locus*	Gene name	SNP[1]	Significance, type and position[2]	Restriction enzyme[3]	GenBank accession number	Reference
SSC1	*ESR1*	estrogen receptor alpha	472 Y	silent exon 8	*Ava*I	AF034974	Drogemuller *et al.* [1997]
SSC1	*ESR1*	estrogen receptor alpha	562 R	silent exon 8		AF034974	Drogemuller *et al.* [1997]
SSC1	*ESR2*	estrogen receptor beta	388 R	M317V	*Hsp*92II	AY357117	Munoz *et al.* [2004]
SSC1	*MC4R*	melanocortin 4 receptor	171 R	I57M	*Hsp*92II	DQ388767	DQ388767
SSC1	*MC4R*	melanocortin 4 receptor	551 Y	F184S	*Bcc*I	DQ388767	DQ388767
SSC1	*MC4R*	melanocortin 4 receptor	758 Y	V253A	*Sau*96I	DQ388767	DQ388767
SSC1	*MC4R*	melanocortin 4 receptor	678 R	D298N	*Taq*I	AF087937	Kim *et al.* [2000a]
SSC1	*IGF1R*	insulin-like growth factor 1 receptor	145 Y	introns 9	*Sac*II	AJ491314	Kopecny *et al.* [2002]
SSC1	*IGF1R*	insulin-like growth factor 1 receptor	152 Y	introns 9	*Bbs*I, *Mbo*II	AJ491314	Kopecny *et al.* [2002]
SSC1	*MYO5A*	myosin VA, myoxin	3071 Y	S1025L	*Hpy*188I	AB209957	Okumura *et al.* [2006]
SSC1	*MYO5A*	myosin VA, myoxin	166 S	T1119S	*Fnu*4HI, *Tse*I	AB222082	Okumura *et al.* [2006]
SSC1	*MEF2A*	myocyte enhancer factor 2A	413 K	silent	*Fnu*4HI	AF053924	Larsen *et al.* [1999]
SSC1	*ADRP*	adipose differentiation related protein	3787 R	G27D	*Hae*III	AY621062	Kim *et al.* [2005]
SSC1	*ADRP*	adipose differentiation related protein	7886 R	G166S		AY621062	Kim *et al.* [2005]
SSC1	*ADRP*	adipose differentiation related protein	10542 Y	R302C	*Tsp*RI	AY621062	Nie *et al.* [2005]
SSC1	*ADRP*	adipose differentiation related protein	11853 R	R333Q	*Sty*I	AY621062	Kim *et al.* [2005]
SSC1	*TGFB1R*	transforming growth factor receptor beta	141 Y	P8S	*Bsp*1286I	AB182258	Shimanuki *et al.* [2005]
SSC1	*TGFB1R*	transforming growth factor receptor beta	2767 R	I413V		AB182258	Shimanuki *et al.* [2005]
SSC1	*Lhx3*	LIM family pituitary transcription factor	330 R	intron 2	*Bsa*HI	AF345446	Smith *et al.* [2001]
SSC1	*ME1*	malic enzyme 1	1762 K	3'UTR	*Tfi*I	X93016	Vidal *et al.* [2006]
SSC2	*TNNT3*	skeletal muscle troponin T3	153 Y	intron 14	*Mln*I	AJ566367	Davoli *et al.* [2003a]
SSC2	*CTSF*	cathepsin F	263 S	D356E	*Bse*RI, *Msl*I	AW670654	Russo *et al.* [2004]
SSC2	*PYGM*	muscle glycogen phosphorylase	55 K	intron 5	*Mln*I	AJ507153	Te Pas *et al.* [2003]
SSC2	*LXRA*	liver X receptor alpha NR1H1	96-97 YS	A32V	*Mwo*I, *Fau*I, *Aci*I	DQ061863	Yu *et al.* [2006]
SSC2	*MYOD1*	myogenic factor 3, MYF3	302 R	5'UTR		U12574	Urbanski and Kurył [2004a]

Table 1. Continued

Chro-mosome	*Locus*	Gene name	SNP[1]	Significance, type and position[2]	Restriction enzyme[3]	GenBank accession number	Reference
SSC2	*MYOD1*	myogenic factor 3, MYF3	566 S	R76P	*Bss*SI	U12574	Urbanski and Kurył [2004b]
SSC2	*IGF2*	insulin-like growth factor 2	16144 R	intron 3	*Fnu*4HI, *Tse*I	AY242112	Van Laere *et al.* [2003]
SSC2	*CAST*	calpastatin	408 R	N167S	*Bfa*I	DQ339697	Ciobanu *et al.* [2004]
SSC2	*CAST*	calpastatin	47 R	R339K	*Hpy*188I	DD217638	Ciobanu *et al.* [2004]
SSC2	*CAST*	calpastatin	1186 S	L396V		NM_214067	Ciobanu *et al.* [2004]
SSC2	*CAST*	calpastatin	1780 R	A594T	*Fnu*4HI	NM_214067	Ciobanu *et al.* [2004]
SSC2	*CAST*	calpastatin	499 M	R728S	*Pvu*II	DD217639	Ciobanu *et al.* [2004]
SSC2	*LDHA*	lactate dehydrogenase	46 K	silent	*Hpy*CH4V, *Bse*YI	AJ557233	Fontanesi *et al.* [2003]
SSC3	*GUSB*	beta glucuronidase	6697 Y	silent	*Hae*II, *Bsa*HI	DQ095863	DQ095863
SSC3	*HUMMLC2B*	skeletal muscul myosin regulator light 2	2744 Y	P144L	*Mnl*I	AY870651	Davoli *et al.* [2003b]
SSC3	*APOB*	apolipoprotein B	6117 S	E1443Q		M22647	Jiang & Gibson [1999]
SSC3	*SULT1A1*	phenol sulfating phenol sulfotransferase 1	76 R	intron 3	*Alu*I	AJ885177	AJ885177
SSC4	*ATP1B1*	ATPase Na K transporting beta 1	1386 Y	intron	*Tsp*RI	AJ401029	Blazkova *et al.* [2000]
SSC4	*APOA2*	apolipoprotein A2	350 R	intron3	*Xma*JI	AJ564196	Knoll *et al.* [2003]
SSC4	*DECR1*	mitochondrial 2,4 dienoyl CoA reductase	90 S	V54L	*Bfa*I	AF335499	Clop *et al.* [2002]
SSC4	*CRH*	corticotropin releasing hormon	400 R	R28Q	*Hpa*II, *Msp*I	AF440229	Wimmers *et al.* [2002]
SSC4	*SDHC*	succinate dehydrogenase subunit C	1611 R	intron5	*Msp*I, *Hpa*II	AJ300475	Stratil *et al.* [2001]
SSC4	*PKLR*	puruvate kinase	384 Y	intron 10	*Mva*I	AJ251197	Knoll *et al.* [2000]
SSC4	*CASQ1*	calsequestrin	144 Y	intron 5	*Alw*26I	AJ488283	Knoll *et al.* [2002]
SSC4	*DGAT1*	diacyloglycerol acetylotransferase	7545 Y	3'UTR	*Msp*I, *Hpa*II	AY116586	Nonemann and Rohrer [2002]
SSC4	*A FABP*	adipocyte fatty acid binding protein	5005 R	intron 1	*Bsm*I	Y16039	Gerbens *et al.* [1998]
SSC4	*MEF2D*	myocyte enhancer factor 2D	638 Y	intron4	*Aci*I	AJ519842	Wagenknecht *et al.* [2003]
SSC5	*MYF5*	myogenic factor 5	65 M	5' flanking	*Aci*I, *Hpy*188I	Y17154	Urbanski and Kurył [2004a]

Table 1. Continued

Chro-mosome	*Locus*	Gene name	SNP[1]	Significance, type and position[2]	Restriction enzyme[3]	GenBank accession number	Reference
SSC5	*MYF5*	myogenic factor 5	580 Y	5' flanking	*Fok*I, *Tsp*45I	Y17154	Urbanski and Kurył [2004a]
SSC5	*MYF6*	myogenic factor 6, herculin	255 Y	5' flanking	*Msp*I	AY327443	Wyszyńska-Koko and Kurył [2004]
SSC5	*MYF6*	myogenic factor 6, herculin	942 M	silent	*Fnu*4HI	AY327443	Wyszyńska-Koko and Kurył [2004]
SSC5	*KITLG*	KIT ligano	97 R	T248A	*Bsl*I, *Hpy*CH4III	AB209961	Okumura *et al.* [2006]
SSC5	*IGFI*	insulin-like growth factor I	155 W	5'UTR	*Mse*I	X52077	X52077 *vs* NM_214256
SSC5	*PTHLH*	parathyroid hormon like hormone	375 Y	S19L		AY193782	Chomdej *et al.* [2004]
SSC6	*LIPE, HSL*	hormone sensitive lipase	433 R	I145V	*Bsa*HI	AJ224692	Knoll *et al.* [1998]
SSC6	*LIPE, HSL*	hormone sensitive lipase	3436 K	E263D		AJ006076	Harbitz *et al.* [1999]
SSC6	*MC5R*	melanocortin 5 receptor	303 R	A109T	*Bsa*HI	AF133793	Kim *et al.* [2000b]
SSC6	*FUT1*	fucosylotranspherase 1,2, alpha	1465 R	R286Q	*Fau*I	U70883	Meijerink *et al.* [1997]
SSC6	*GYS1*	glycogen synthase	418 R	intron 14		AJ507152	Te Pas *et al.* [2003]
SSC6	*TGFB1*	transforming growth factor beta	180 R	intron 6	*Ava*II	AJ621785	Kopecny *et al.* [2004]
SSC6	*LEPR*	leptine receptor	609 Y	T69M		AF184173	Mackowski *et al.* [2005]
SSC6	*LEPR*	leptine receptor	620-621 WY	S73I	*Tsp*509I	AF184173	Mackowski *et al.* [2005]
SSC6	*LXRB*	liver X receptor beta NR1H2	147 Y	silent	*Aci*I	DQ060239	Yu *et al.* [2006]
SSC6	*RYR1*	ryanodine receptor	1666 Y	R615C	*Hha*I, *Alw*21I	X68247	Fujii *et al.* [1991]
SSC6	*H FABP*	heart fatty acid binding protein	1324 Y	5' flanking	*Hinf*I	X98558	Gerbens *et al.* [1997]
SSC6	*H FABP*	heart fatty acid binding protein	737 Y	I51T	*Hph*I	Y16180	Gerbens *et al.* [1997]
SSC7	*Hsp70*	heat shock protein	266 S	5' flanking	*Fnu*4HI	AJ309021	Schwerin *et al.*[2001]
SSC7	*CBG*	cortycosteroid binding globulin	198 K	5' flanking		AF510490	AF510490
SSC7	*PSME1*	proteasome activator PA28α	560 Y	intron 8	*Sph*I	AY177614	Wang *et al.* [2004]
SSC7	*CYP21*	steroid 21 hydroxylase	2991 M	intron splicing site	*Nla*IV	M83939	Knoll *et al.* [1998]

Table 1. Continued

Chro-mosome	*Locus*	Gene name	SNP[1]	Significance, type and position[2]	Restriction enzyme[3]	GenBank accession number	Reference
SSC7	*GNMT*	glycine N methylotrasferase	99 R	unknown	*Hpy*188I	D13308	Ponsuksili *et al.* [2005]
SSC7	*PKM2*	puruvate kinase 2 muscle	32 Y	3'UTR	*Aci*I	AJ557235	Fontanesi *et al.* [2003]
SSC7	*PRL*	prolactin	117 Y	silent		AY905690	Korwin-Kossakowska *et al.* [2006]
SSC8	*GNRHR*	gonadotropin releasing hormone receptor	593 Y	5'UTR	*Hpy*CH4III	AF227685	Jiang *et al.* [2001]
SSC8	*PPARGC1*	peroxisome proliferator activated receptor gamma coactivator1	678 W	C430S	*Alu*I	AY484500	Kunej *et al.* [2005]
SSC8	*LDLRRP1*	low density lipoprotein receptor related protein1	459 R	3'UTR	*Alu*I	AF526393	AF526393
SSC8	*STK32*	serine/threonine protein kinase	763 Y	STS	*Alu*I, *Fnu*4HI	BV102996	Kim *et al.* [2004]
SSC8	*CLNG*	calmegin	169 Y	intron	*Hind*III, *Alu*I	AY536213	Kim *et al.*[2004]
SSC9	*HSD11B1*	beta hydroxysteroid dehydrogenase	446 S	Q123H	*Stu*I	AF414124	Otieno *et al.* [2005]
SSC9	*MYOG*	myogenin	673 Y	silent	*Sex*AI	X89007	Wyszynska-Koko and Kurył [2005]
SSC9	*TYR*	tyrosinase	663 Y	silent	*Msc*I	AB207236	Okumura *et al.* [2005]
SSC9	*SLN*	sarcolipin	235 R	3'UTR	*Bst*BI, *Taq*I	Z98820,	Davoli *et al.* [1999]
SSC9	*SDHD*	succinate dehydrogenase D	444 K	silent		AY682832	Zhu *et al.* [2005]
SSC10	*PPKCQ*	protein kinase C theta	171 Y	3'UTR	*Nla*III	AF473820	Nonneman and Rohrer [2003]
SSC10	*AKR1C2*	aldo keto reductase 1	206 R	introns	*Hae*III	AF473815	Nonneman and Rohrer [2003]
SSC10	*GAD2*	glutamate decarboxylase 2 gene	340 Y	intron	*Bts*I	AF473817	Nonneman and Rohrer [2003]
SSC10	*ATP5C1*	ATP synthase gamma subunit1	235 R	intron	*Mfe*I	AF473816	Nonneman and Rohrer [2003]
SSC11	*EDNRB*	endothelin receptor type B	344 Y	P64S	*Cac*8I, *Bsm*AI	AB209960	Okumura *et al.* [2006]
SSC11	*TYPR2*	dopachrome tautomerase	975 M	L184F		AB207241	Okumura *et al.* [2005]
SSC11	*TYPR2*	dopachrome tautomerase	1843 Y	P474S	*Bsr*I	AB207241	Okumura *et al.* [2005]
SSC11	*ESD*	esterase D	587 R	E196G	*Bcc*I	AB032555	Omi *et al.* [2000]
SSC12	*GH*	growth hormone	200 K	SP1 binding	*Mln*I	U58113	Larsen and Nielsen [1997]

Table 1. Continued

Chro-mosome	*Locus*	Gene name	SNP[1]	Significance, type and position[2]	Restriction enzyme[3]	GenBank accession number	Reference
SSC12	*GH*	growth hormone	306 W	TATA box		U58113	Larsen and Nielsen [1997]
SSC12	*GH*	growth hormone	485 R	R22Q	*Dde*I	AY727040	Kirkpatrick *et al.* [1993]
SSC12	*GH*	growth hormone	494-495 SM	G25Q	*Hae*III	AY727040	Kirkpatrick *et al.* [1993]
SSC12	*MYH4*	myosin heavy chain 2B	26 W	3'UTR	*Hpy*F44III	AJ493461	Davoli *et al.* [2003]
SSC12	*STAT5a*	signal transducer and activator of transcription 5A	643 Y	C197R	*Hga*I	AF135122	AF135122
SSC12	*STAT5a*	signal transducer and activator of transcription 5A	1424 Y	F457S	*Bsa*JI	AF135122	AF135122
SSC12	*STAT5a*	signal transducer and activator of transcription 5A	2185 Y	F711L	*Alu*I	AF135122	AF135122
SSC12	*STAT5a*	signal transducer and activator of transcription 5A	2435 Y	T794I	*Bsa*JI	AF135122	AF135122
SSC12	*STAT5b*	signal transducer and activator of transcription 5B	470 Y	P153L	*Hpa*II	AF135123	AF135123
SSC12	*STAT5b*	signal transducer and activator of transcription 5B	1145 R	R378Q		AF135123	AF135123
SSC12	*STAT5b*	signal transducer and activator of transcription 5B	1187 R	C392Y	*Fnu*4HI	AF135123	AF135123
SSC12	*FASN*	fatty acid synthetase	265 Y	silent	*Fnu*4HI	AY183428	Munoz *et al.* [2003]
SSC12	*FDXR*	ferrodoxin reductase	562 S	intron	*Hha*I	AF317683	Yu *et al.* [2003]
SSC12	*GAA*	alpha acid glucosidase	38 Y	silent	*Taq*I	AJ557226	Fontanesi *et al.* [2003]
SSC12	*ESTL147*	*EST*	189 Y	unknown	*Hpy*188I	BF713799	Ponsuksili *et al.* [2005]
SSC13	*PIT1*	Pit1 transcrition factor	243 R	N152D		U00793	Yu *et al.* [1994]
SSC13	*PIT1*	Pit1 transcrition factor	1141 R	R215G		U00793	Yu *et al.* [1994]
SSC13	*BMP15*	bone morphogenic protein	6009 R	E20G	*Bss*KI	AF458070	Wang *et al.* [2003]
SSC13	*SIAT1*	A2,6 sialyltransferase	191 Y	unknown	*Fok*I	AF136746	Vedrine and Mouricout [1998]

Table 1. Continued

Chro-mosome	*Locus*	Gene name	SNP[1]	Significance, type and position[2]	Restriction enzyme[3]	GenBank accession number	Reference
SSC13	*HGD*	homogentisade oxydase	354 Y	P>S	*Ava*II	BE232117	Ponsuksili *et al.* [2005]
SSC13	*PPARG*	peroxisome proliferator activated receptor gamma 1	324 R	5' flanking		AY044238	Grindflek *et al.* [2004]
SSC13	*STCH*	stress 70 protein ATPase	167 Y	unknown		AF513506	Shi *et al.* [2002]
SSC13	*CSTB*	cystatin	335 R	Q52R	*Alu*I	AJ315561	Russo *et al.* [2002]
SSC13	*CSTB*	cystatin	367 R	D63N		AJ315561	Russo *et al.* [2002]
SSC14	*CTSB*	cathepsin B	162 Y	intron	*Bse*AI	AJ315558	Russo *et al.* [2002]
SSC14	*CYP2E1*	cytochrome p 450 2E1	2412 Y	5' flanking		AJ697882	Skinner *et al.* [2005]
SSC14	*CYP2E1*	cytochrome p 450 2E1	744 R	A475T	*Nru*I	AJ697884	Skinner *et al.* [2005]
SSC14	*SCD*	stearoyl CoA desaturase	2228 Y	5' flanking	*Psy*I	AY487830	Ren *et al.* [2004]
SSC14	*MAPK8*	mitogen activated protein kinase 8	258 Y	intron		AF473819	Nonneman and Rohrer [2003]
SSC14	*PRKAB1*	protein kinase AMP activated beta	191 R	intron	*Alu*I	AJ557221	Fontanesi *et al.* [2003]
SSC14	*MYOP*	myopalladin	298 K	3'UTR	*Hpy*CH4V	AJ560657	AJ560657
SSC14	*LPL*	lipoprotein lipase	1026 R	intron6	*Sma*I	AY332511	Lei *et al.* [2004]
SSC14	*PRSS11*	protease serine 11 [IGF binding]	8245 R	5' flanking	*AclI*, *Hpy*CH4IV	AJ853849	Haase *et al.* [2005]
SSC14	*TCF1*	hepatic nuclear factor 1	314 Y	intron		AJ885176	AJ885176
SSC15	*PRKAG3*	AMP activated protein kinase γ subunit	89 M	T80N	*Sty*I	AF214521	Ciobanu *et al.* [2001]
SSC15	*PRKAG3*	AMP activated protein kinase γ subunit	514 R	G102S	*Bsl*I	AF214521	Ciobanu *et al.* [2001]
SSC15	*PRKAG3*	AMP activated protein kinase γ subunit	518 Y	L103P	*Bmr*I	AF214521	Ciobanu *et al.* [2001]
SSC15	*PRKAG3*	AMP activated protein kinase γ subunit	1845 R	V249I	*Bsa*HI	AF214521	Milan *et al.* [2000]
SSC15	*PRKAG3*	AMP activated protein kinase γ subunit	1849 R	R250Q	*Bsr*BI	AF214521	Milan *et al.* [2000]
SSC15	*MSTN*	myostatin	2150 Y	silent	*Taq*I, *Hinf*I	AJ237920	Stratil and Kopecny [1999]
SSC15	*MSTN*	myostatin	607 W	5' flanking	*Dra*I	AJ133580	Stratil and Kopecny [1999]
SSC15	*TTN*	titin	137 Y	3 UTR		AJ560658	AJ560658
SSC15	*DES*	desmin	749 Y	silent		AF136188	Tuggle *et al.* [1999]
SSC15	*INHA*	α inhibin	184 R	silent	*Hin*6I	AY028465	Hiedleder *et al.* [2002]

Table 1. Continued

Chro-mosome	*Locus*	Gene name	SNP[1]	Significance, type and position[2]	Restriction enzyme[3]	GenBank accession number	Reference
SSC16	*PRLR*	prolactin receptor	201 R	S591G	*Msp*I, *Ngo*MIV	U96306	Vincent *et al.* [1997]
SSC16	*GHR*	growth hormone receptor	155 R	silent R51		DQ388035	DQ388035
SSC17	*SFRS1*	splicing factor arginine/serine rich 1	1146 Y	intron	*Hpy*188III, *Msp*I	DQ098951	Wang *et al.* [2005]
SSC17	*AHCY*	S adenosylo homo cystein hydralase	83148 R	5' flanking		AJ427478	Leeb *et al.* [2000]
SSC17	*MCR3*	melanocortin 3 receptor	522 Y	silent	*Mnl*I	AJ744762	Civanova *et al.* [2004]
SSC18	*LEP*	leptine	3469 Y	silent	*Hinf*I	U66254	Stratil *et al.* [1997]
SSC18	*PGAM2*	phosphoglycerate mutase 2	77 M	silent	*Hin*P1I, *Hha*I	AJ557237	Fontanesi *et al.* [2003]
SSCX	*QTL BamHI*	QTL RFLP marker	94 Y	QTL marker	*Bam*HI, *Bsp*HI	AY574041	Gaboreanu *et al.* [2004]
SSCX	*AR*	androgen receptor	154 K	silent exon 1		AB052938	Shimanouki *et al.* [2001]
unknown	*ADD1*	adipocyte determination and differentiation factor	424 R	intron		AY272051	AY272051
unknown	*APM*	adiponectin	1783 Y	unknown	*Acc*I	AY627882	AY627882
unknown	*CYP4A24*	fatty acid hydroxylase	2505 Y	unknown	*Hha*I	AJ586619	Lundell [2004]
unknown	*FHL3*	four and half LIM only protein	312 R	G75R	*Pst*I	AY377857	Zuo *et al.* [2004]
unknown	*CYP2A6*	cytochrom p450 2A6	454 indel G	frame shift		AY091516	Lin *et al.* [2004]

[1]Position of a SNP according to GenBank numeration.
[2]Missense mutations denoted with position of a substitution in the polypeptide chain and alternative amino acid variants; A – alanine; R – arginine; N – asparagine; D – aspartic acid; C – cysteine; Q – glutamine; E – glutamic acid; G – glycine; H – histidine; I – isoleucine; L – leucine; K – lysine; M – methionine; F – phenylalanine; P – proline; S – serine; T – threonine; W – tryptophan; Y – tyrosine; V – valine; UTR – untranslated region.
[3]Restriction enzyme potentially recognizing polymorphism in PCR-RFLP assay.

Information on allele frequency is also useful in planning population experiments. If an allele is very rare or specific for uncommon breed it should be eliminated because of the low probability of finding a genotype group of animals for associated studies. Therefore, alleles occurring in rare (endangered) pig breeds were excluded from the database. SNPs were catalogued mostly of major pig breeds (e.g. Large White, Landrace, Pietrain, Duroc) because of their economic importance.

The reason for the current vital interest in SNPs is the hope that they could be used as markers to identify genes associated with multifactoral disorders or quantitative trait *loci* (QTLs) – Coronini *et al.* [2003]. It is assumed that SNP alleles are inherited together with QTLs over generations because they are physically close to each other. In contrast to microsatellite markers, SNPs are frequently dispersed throughout the genome and therefore can be used for QTL fine mapping. The rationale would be to genotype a collection of SNPs that occur at regular intervals and cover the whole genome to detect genomic regions in which the frequencies of the SNP allele differ between experimental populations. The genome-wide SNP genotyping is theoretically possible for the human genome for which over 2 million SNPs are available in the public database (SNPdb, National Center of Biotechnological Information, USA). The throughput required for genotyping even some of the thousands of SNPs and the current cost of genotyping makes such projects impractical. A more feasible alternative to random whole-genome SNP mapping is to use SNP markers in candidate genes which are thought to be associated with certain QTLs. This is the only choice for genomes for which no dense SNP database has been published, but have numerous detected SNPs dispersed in many publicly available sources.

In pig genome, more than 1500 SNPs were recently identified and deposited in the public domain [Fahrenkrug *et al.* 2002]. In this case the SNPs were obtained from ESTs collections based on Western and Chinese breeds without description of functional significance of a SNP.

Our database is focused specifically on a narrow set of SNPs with known function. Surely, the information on polymorphism will be steadily growing and therefore should be arranged and formatted to better design and interpret future experiments with the use of high throughput screening techniques. In case of pig SNPs there is an evident lack of uniform information on the topic. In papers, SNPs are described mostly at the protein level as an amino-acid change with or without relevant nucleic acid sequence information. In contrast, in the GenBank database, sequences are not annotated sufficiently (location and type of SNP) or dispersed within different records. Before using these sequences for multi-*loci* genotyping they must be "manually" analysed. To avoid this limitation, in the present paper selected available sequence and research information have been gathered to create well-organized database of SNPs described in the same format.

Our general strategy was to collect SNPs potentially involved in pork traits formation, but other polymorphisms were included as they are involved in basic

biochemical processes in the muscle tissue or play a fundamental role in the functioning of the whole organism.

Whether all known SNPs within one gene should be included in the database is open to criticism. The more SNPs within a *locus*, the more options are available to design effective primers or probes. However, too many synonymous SNPs or repeats within one *locus*, which are indirectly or only potentially associated with a phenotype seem useless and, in the authors' opinion, should be ignored. On the other hand, the reduction of a number of SNPs may lead to missing an interesting genetic phenomenon – interacting phenotype effects of co-existing variants located within the 5'- and 3'- flanking regions of a single gene [Schwerin *et al.* 2002]. Therefore, a pre-selection was made from the *loci* containing many SNPs. Only non-synonymous mutations, mutations involved in gene expression events or variants most frequently occurring in breeding populations were included. Because very short stretches of DNA are inconvenient or even useless in primer design, all sequences shorter than 25 bp were excluded from the database.

The database contains SNPs determining four major genes (or QTLs) – *RYR1, PRKAG3, CAST* and *IGF2*.

A separate group of gene polymorphisms associated with production and quality of pork occurs within the microsatellite markers [Geldermann *et al*. 2003]. These QTL markers were excluded from the database presented here for three reasons: (i) the nature of their polymorphism is often unclear (the type of repetitive motif, its location and number of repeats), (ii) repetitive sequences are difficult to genotype by primer extension reaction (which is the most often used method in high throughput genotype screening on a chip), and (iii) the most microsatellite markers share approximately 10 alleles, that makes genotyping more expensive.

A way to include these genomic regions into a DNA chip is sequencing the regions located around a QTL and then comparing these sequences from a population of animals to find new biallelic SNPs. The probability of finding a SNP could be lower than in humans (1/1250 bp) because of the higher homogeneity of commercial lines of pigs. Such SNPs may substitute microsatellite markers to enable their implementation in high throughput genotyping.

The database should be continuously updated by new data, and a potential source of new SNPs are pig muscle expressed sequence tags (ESTs).

The primary application of this SNP database is designing a chip for the simultaneous genotyping of many SNPs present in candidate genes to reveal the genetic background of production and quality of pork. Particularly, meat quality is one of the most important criterions in pig selection, and will soon be reflected in pork pricing. It is believed that a combination of SNPs will be found acting as very effective set of genetic markers in selection for productive performance in pigs.

In several genes, mutations were catalogued which create intragenic haplotypes (many SNPs within one gene), e.g. *CAST, HFABP, PRKAG3, GH*.

The collected SNPs represent all of the 18 pig chromosomes. Most of these SNPs may play a role as markers of certain chromosome regions, while others should be treated as functional mutations affecting different traits. Because genes affecting certain traits are sometimes organized in groups and located close together due to co-expression, the SNPs described in the catalogue may turned to be more efficient genetic markers and may shorten the way to find neighbouring mutations influencing both production performance traits in pigs and quality of pork.

Another possible application of the SNP database is pig identification and parental control. Compared with the most popular DNA markers (microsatellites), SNPs are attractive because of their abundance, genetic stability and amenableness to high-throughput automated technology [Vignal 2002]. They are considered as a realistic alternative in livestock identification and kinship analysis [Fries and Durstewitz 2001, Heaton *et al.* 2002]. Before it, however, a wide population screening must be conducted to validate the frequency of SNPs in major commercial pig breeds. The SNPs database can also be used to develop a genetic traceability tests [Goffaux *et al.* 2005], as well as for evolutionary studies, evaluation of genetic distances between wild and domestic pig breeds (lines) and domestication history of *Sus* species.

Although the SNPs database contains only a part of all existing variation associated with pork yield and quality, its originality, as well as current and future applicability make it a valuable resource for designing various experiments, especially for studying SNPs interactions with the use of microarray technology.

An MySQL version of presented database is under construction and development and will be soon publicly available from the University of Warmia and Mazury server.

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Baza polimorficznych sekwencji nukleotydowych w obrębie genów kandydujących, potencjalnie związanych z produkcją i jakością wieprzowiny

S t r e s z c z e n i e

Rosnąca liczba mutacji w obrębie genów związanych z produkcją i jakością wieprzowiny nie została dotąd sklasyfikowana i opisana w sposób umożliwiający projektowanie eksperymentów i ich interpretację metodą multiplex PCR lub wysokowydajnymi technikami przesiewowymi. Celem podjętej pracy była obróbka i skatalogowanie publicznie dostępnych informacji o polimorficznych sekwencjach nukleotydowych *(single nucleotide polymorphisms* – SNPs) położonych w genach bezpośrednio, pośrednio lub potencjalnie związanych z produkcją wieprzowiny i jej jakością.

Skonstruowano bazę zawierającą 153 sekwencje (SNPs) w obrębie 113 genów. Wszystkie polimorfizmy opisano w sposób, który umożliwia automatyczne pobieranie sekwencji w formatach dostępnych w GenBank do specjalistycznego oprogramowania służącego jednoczesnemu projektowaniu wielu starterów PCR oraz allelo-specyficznych sond używanych w technologii mikropłytek (*microarray technology*).

Przedstawiony zbiór sekwencji SNPs może służyć jako wiarygodne źródło do studiów nad interakcjami między wybranymi SNPs, prowadzącymi do wyjaśnienia genetycznego podłoża zmienności umięśnienia świń, a po przeprowadzeniu szerokich badań populacyjnych także do kontroli pochodzenia i badań filogenetycznych trzody chlewnej.