

USE OF MODERN GENETICS ACHIEVEMENTS FOR IMPROVEMENT OF PORK QUALITY – A REVIEW*

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According to the FAO database [2002], approximately 40% (94 million metric tons) of the red meat consumed annually worldwide is pork. Pork consumption has been increasing consistently with the increase of world population. In the past decade, modern research achievements towards genetic improvement of economic traits, like growth rate, based on studies of myogenesis and metabolomics of adipose tissue, have had a major impact on improving the carcass composition, meat quality and efficiency of the pork production (in swine industry). These technologies based on research in functional genomics, have a significant potential, but considerable research effort will be required before they can effectively be utilized in pig production. Knowledge about the sequence of the pig genome would help to identify new candidate genes and unique regulatory elements. This great promise provides new information about regulation of expression of such genes that can be used to enhance efficiency of pork production in the future. The aim of this study was to assess a comprehensive overview on functional candidate genes related especially to myogenesis, for examples: growth hormone (*GH*), growth hormone receptors (*GHR*), growth hormone realizing hormone (*GHRH*), growth hormone realizing hormone receptors (*GHRHR*), insulin like growth factors and their receptors (*IGF*, *IGF-I*, *IGF-II*, *IGF-IR*), pituitary-specific transcription factor 1 (*PIT-1* renamed as *POU1F1*), leptin (*LEP*), leptin receptors (*LEPR*), myogenic regulatory factors gene family (*MRF*), the protein kinase adenosine monophosphate-activated γ 3-subunit (*PRKAG3*) and the melanocortin receptor gene family (*MCR*), for body growth rate and carcass composition traits towards their functional role for the genetic improvement of meat quality and efficiency of the pork production.

INTRODUCTION

Investigations based on the possibility of predicting the improved prognosis of the growth rate and carcass and meat quality in farm animals are recently one of the main problems and challenge in animal genetic studies. A great progress in molecular genetics as well as functional genomics allows to expect the identification of genes to have a significant effect on productive traits in the nearest future. The identification of *RYR1* gene mutation in pig, known earlier as the “halothane” gene, was a great achievement in this field of scientific studies. Elimination of this gene from pig populations resulted in the improvement of meat quality, as PSE meat was eliminated simultaneously. However, other defects of technological quality of pork appeared, e.g. high drip loss caused by mutation in *PRKAG3* (the adenosine monophosphate-activated protein kinase) gene, firstly known as hypothetic RN^+ / RN^- gene variants. The genetic studies are aimed at identifying gene mutations, resulting from selection. It is known that the improvement of one trait may lead to an unprofitable level of another one. A high growth rate and carcass meatiness in pigs were a beneficial result of selection carried out for a number of years, still they simultaneously deteriorated meat quality. The selection-related changes in muscle microstructure were, in turn, reported to affect technological properties of meat.

In the last decade, investigations concentrated on genes or gene families of known function contributing significantly

to genetic improvement of the instance growth rate, muscle development (myogenesis), and metabolism of adipose tissue. A group of genes coding for growth factors, their receptors, transporting and regulatory proteins seemed to be an attractive subject of similar studies.

Growth hormone (GH) and insulin-like growth factors (IGFs) are of major significance to the regulation of body growth and composition. They are also important for processes of cell proliferation and differentiation. Selection aimed at improving lean meat content of carcass and decreasing fat thickness resulted in changes in the concentration of GH and IGF in blood plasma. GH regulates the expression of *IGF* gene in liver and fat tissue but not in the muscle. An interaction of factors of GH-IGF-I axis also includes receptors for GH and IGF-I and numerous binding proteins. It was shown experimentally that administration of exogenous growth hormone to finishing pigs increased growth rate and muscle hypertrophy, reduced fat deposition, decreased the number and size of adipocytes, reduced the activity of lipogenic enzymes and insulin responsiveness of adipocytes and increased lipolysis rate. The mechanisms underlying these relationships remain unclear and detailed analyses are needed in this respect [Te Pas *et al.*, 2004].

It should be mentioned that long-term excess of GH leads to acromegaly, a disease which is associated with the myopathy in which the muscles are hypertrophic but functionally weaker [Weber, 2002]. Moreover, an interaction of growth factors with other hormones and factors affects growth rate

and body composition, for instance, the pituitary-specific transcription factor 1 (PIT-1) regulating *GH* mRNA expression or *IGF-I* affecting myogenin expression. Myogenin belongs to myogenic regulatory factors (MRFs) controlling the myogenesis processes. It was concluded that the selection for increased growth rate was associated with increased mRNA levels of the *MRF* genes expressed in satellite cells [Te Pas *et al.*, 2004].

The effect of GH on lipid metabolism is well documented in pigs. Exogenous GH treatment decreases lipid deposition regardless of sex, breed or age. It was concluded that reduction in lipid deposition resulted from a decrease in lipogenesis rather than from an increase in lipolysis, and involved a decrease in adipocyte insulin sensitivity. The mechanism at the cellular level is unresolved, however, *in vitro* and *in vivo* experiments documented those relations [Louveau & Gondret 2004].

In vivo experiments showed the importance of IGF factors in muscle development. The time-course of *IGFs* expression during embryo development is related to fiber formation in pigs. The expression of *IGF-I* and *IGF-II* mRNA increases from 44 to 59 day of embryo development - period of primary fiber formation. Moreover, *IGF-I* mRNA further increases from 75 day - period of secondary fiber formation, whereas *IGF-II* mRNA decreases steadily. The steady decrease of *IGF-II* mRNA may be due to the elevated level of *IGF-I* mRNA, which inhibits IGF-II production. An increase in the expression of *IGF-I* mRNA following GH treatment was dependent on a muscle and was found in *M. semitendinosus* but not in *M. longissimus*. A high number of type I IGF- receptors has been found in membrane from slow-twitch muscles compared to fast-twitch muscles. It partly may explain a direct influence of IGF-I on muscle hypertrophy [Oksbjerg *et al.*, 2004]. These relationships affecting muscle microstructure influence meat quality *post mortem*.

GROWTH HORMONE (*GH*) GENE

The results presented in several studies carried out in 1990-ies suggested that an association between polymorphism of growth hormone gene and carcass traits of different commercial breeds and lines should be analysed. It might evidence the usefulness of particular *GH* gene variant as a marker in pig selection [Knorr *et al.*, 1997]. Similar studies were undertaken in Poland. Mapping of genes affecting meat and fat deposition in pig carcass was a subject of project realized by several collaborating laboratories. Among other things, the QTL (quantitative trait locus) for abdominal fat content was identified [Korwin-Kossakowska *et al.*, 2001]. That hypothetical gene was localized on chromosome 12 in the region comprising locus GH. Thus, studies was undertaken into the association between growth hormone gene polymorphism and carcass quality. An investigation was realized on material comprising 266 castrates of F₂ generation (the reference family originated from a mating of Polish Large White sows and Zlotnicka Spotted boars). A significant effect of *GH* gene mutations, present in exon 2 and intron 2 of this gene, on back fat thickness measured in different points as well as on loin eye area and meat content of carcass was identified [Pierzchała *et al.*, 1999]. A similar relationship was described earlier by German researchers [Knorr *et al.*, 1997] and their reference family originated from a mating of Pietrain sows

and Meishan boars. A value of majority of traits characterising fat deposition in carcass was dependent on *GH* genotype.

Several different breeds and crosses reared in Poland were also analysed. A significant effect of *GH* genotype on weight of sirloin was observed among Torhyb crosses [(Polish Large White x Polish Landrace) x Pietrain], whereas a highly significant one on the weight of ham and ham meat among PIC pigs [Kurył *et al.*, 2003]. A similar effect of the *GH* genotype on carcass traits was observed within 322 crosses (Table 1). They originated from the mating of sows being crosses of Polish Large White and Polish Landrace breeds and boars of breeds as follows: Polish Landrace, Polish Large White, Duroc, Pietrain. A significant or highly significant effect of *GH* genotype on the following traits was shown: weight of ham, ham meat and bones, share of ham weight in the weight of carcass [Pierzchała *et al.*, 2004a].

Summarizing the results presented in literature one should conclude that a significance of *GH* genotype effect on a value of particular carcass trait differs depending on breed or line. It is well known that quantitative traits (phenotypes) are manifested as a result of additive effects of different genes, which may contribute to the phenotype to a different extent. They are also affected by environmental factors. Therefore, the usefulness of individual gene variant as a marker for particular carcass trait may differ between breeds. One should not expect that any *GH* gene variant would be a versatile marker for carcass meat or fat deposition traits in various pig breeds. One should take into consideration that a significant association between *GH* genotype and a level of carcass fatness was observed only in crosses originated from Zlotnicka Spotted breed which is known as fat pig [Pierzchała *et al.*, 1999]. A similar relationship has been noticed by Knorr *et al.* [1997] but only within animals of F₂ generation originating from mating of Pietrain sows and Meishan boar. No significant associations were found in the Wild Boar x Pietrain family. However, taking into consideration the important role of growth hormone in the lipid metabolism [Louveau & Gondret, 2004], one may expect that a certain variant of *GH* gene may display higher usefulness as a marker in selection directed to decreasing carcass fatness comparing to another variants of this gene. A mutation in the regulatory region of the gene may influence its transcription level and cause higher plasma GH concentration. Nielsen *et al.* [1995] identified a TATA-box polymorphism in a regulatory region of the gene *GH*, which suggested differences in the transcriptional activity between variants of this gene, which may eventually cause higher plasma GH concentration and higher growth rate. However, their study has not established a direct cause-and-effect relationship. Further studies concerning another parts of regulatory region of *GH* gene are needed.

GROWTH HORMONE RECEPTOR (*GHR*) GENE

Polymorphism of the porcine growth hormone receptor gene has not been reported till now. Our own studies aimed at finding any mutation in the porcine gene encoding growth hormone receptor were based on sequencing of the chosen fragment amplified in the PCR reaction. This sequencing performed for several pigs allowed identification of a silent mutation (SNP - single nucleotide polymorphism) in posi-

tion 1266 of transcribed mature mRNA of this gene identified by *KspA1 endonuclease*. This mutation, as a silent mutation, does not change amino acid sequence of the coded protein and for this reason it may not directly influence the level of performance traits. Nevertheless, it could be interesting when it is linked with a causal mutation. An association between *GHR* genotype and performance traits was analysed on the material comprising 322 porkers originating from the mating of sows being crosses of Polish Large White and Polish Landrace breeds and boars of breeds as follows: Polish Landrace, Polish Large White, Duroc, Pietrain. The only significant relation obtained was that between *GHR* genotype and growth rate (Table 1).

GROWTH HORMONE RELEASING HORMONE (*GHRH*) GENE

Growth hormone releasing hormone gene is one of the candidate genes for growth rate and carcass quality in pigs due to the role of its product in secretion of growth hormone. Polymorphism of the porcine *GHRH* gene was identified by Baskin & Pomp [1997]. Studies performed on the reference family originating from the mating of Pietrain sows and Meishan boars allowed identification of QTLs for carcass length and body weight at slaughter in the region of localization of *GHRH* gene on chromosome 17 [Pierzchała *et al.*, 2003a]. There were

not presented studies on a relation between *GHRH* genotype and performance traits in pigs in the literature till now. In our own studies, performed on the material comprising 322 porkers originating from the mating of sows being crosses of Polish Large White and Polish Landrace breeds and boars of breeds as follows: Polish Landrace, Polish Large White, Duroc, Pietrain, an association between genotype at the *GHRH* locus and fat thickness over the shoulder and meat content of ham has been evidenced [Pierzchała *et al.*, 2003b] (Table 1). Further studies on commercial breeds or lines are needed in order to confirm or exclude the usefulness of *GHRH* genotype in selection improving growth rate and carcass quality.

GROWTH HORMONE RELEASING HORMONE RECEPTOR (*GHRHR*) GENE

The gene encoding the growth hormone releasing hormone receptor (*GHRHR*) is one of the crucial components involved in regulation of transcription and release of growth hormone and because of that it seems to be an interesting candidate gene for performance traits in pigs. Own studies undertaken in order to find the polymorphism in this gene resulted in identification of a point mutation (SNP) identified with *PstU1 endonuclease* as well as a composed microsatellite sequence in the 5' flanking region of the *GHRHR* gene. These mutations were localized close to a DNA sequence specific

TABLE 1. An association between the polymorphism of some growth factors and their receptors genes and performance traits in pigs – recapitulation of the results of own studies.

Trait	<i>POU1F1</i>	<i>GH</i>	<i>GHRH</i>	<i>GHRHR</i>	<i>IGF2</i>	<i>GHR</i>
Average daily gain during fattening	**			*		*
Fat thickness						
– over the shoulder		**	*		*	
– on back	*	*				
– at sacrum	**	*		*		
– mean from 5 measurements	*	*				
Ham						
– weight of cut		**		*		
– weight of meat		**		*		
– weight of fat	*	*	*	*		
– meat content	*		*	*		
– fat content						
– weight of bones	*	**				
– share in weight of carcass		**				
Loin						
– weight of cut		*				
– weight of meat						
– weight of fat						
– meat content						
– share in carcass weight						
– eye area		*				
Weight of sirloin		*				
Meat content of carcass	*	*		*		
Carcass length		**				

* – significance at $p \leq 0.05$; ** – significance at $p \leq 0.01$

for binding the pituitary transcription factor 1 (*PIT-1*) which was localized 9 kb upstream from start of transcription of *GHRH* gene. Thus, potentially they could regulate the rate of this gene transcription.

An association between genotype at the *GHRHR* locus and performance traits was analysed on the material comprising 322 porkers originating from the mating of sows being crosses of Polish Large White and Polish Landrace breeds and boars of the following breeds: Polish Landrace, Polish Large White, Duroc, Pietrain (Table 1). A significant relation was observed between the variant of microsatellite sequence in the regulatory region of this gene and meat content of ham and carcass, weight of meat and fat in ham as well as back fat thickness. The SNP identified in the same region of *GHRHR* gene significantly affected weight of ham meat [Pierzchała *et al.*, 2004b].

PITUITARY-SPECIFIC TRANSCRIPTION FACTOR 1 (*PIT-1*, RENAMED AS *POUIF1*) GENE

The POU domain class 1 transcription factor 1 (*POUIF1*) gene encoding the pituitary-specific transcription factor 1 (*PIT-1*) may be interesting as a candidate gene for performance traits in pigs. The *PIT-1* factor plays an essential role in the transcription of the growth hormone, prolactin and thyrotropin β subunit. The *POUIF1* gene is localized on chromosome 13 in the region where QTL for growth rate has been mapped [Andersson *et al.*, 1994]. Therefore, this gene has been accepted as a subject of studies undertaken in order to find markers for growth rate and carcass quality traits. Investigations performed at the Iowa State University showed that body weight of piglets at birth, back fat thickness and loin eye area were significantly related to the *POUIF1* genotype [Yu *et al.*, 1995]. Czech scientists also presented evidence that back fat thickness in crosses of Large White and Landrace breeds was significantly dependent on *POUIF1* genotype [Stancekova *et al.*, 1999].

Our own studies performed on the material comprising 199 castrates of F₂ generation, originating from the mating of Polish Large White sows and Zlotnicka Spotted boars, showed that fat thickness at *sacrum* (point K3) and over the loin as well as mean fat thickness from five measurements and weight of bacon with ribs were dependent on *POUIF1* genotype [Kurył & Pierzchała, 2001]. Next, a significant effect of *POUIF1* genotype on fat thickness in several points of measurements (at *sacrum*, over the loin) was confirmed among 322 crosses originating from the mating of sows being crosses of Polish Large White and Polish Landrace breeds and boars of breeds as follows: Polish Landrace, Polish Large White, Duroc, Pietrain (Table 1). Moreover, daily gain during the fattening period and meat content of carcass and ham were significantly or highly significantly related to *POUIF1* genotype [Pierzchała *et al.*, 200b] (Table 1).

The investigations performed by the German researchers on the material comprising 310 fatteners of F₂ generation, originating from the mating of Pietrain sows and Wild Boar, and another 310 F₂ animals originating from Pietrain \times Meishan crosses showed a significant association between *POUIF1* genotype and carcass traits only in the first group of pigs. Body weight at slaughter, length of carcass, weight of shoulder meat and fat, weight of abdominal fat and feed conversion ratio were related to the *POUIF1* genotype [Brunsch *et al.*, 2002].

Summarizing data presented in literature on the usefulness of *POUIF1* gene variants in prediction of pig carcass quality one may conclude that they do not seem to be universal markers for all pig breeds, which was also concluded concerning *GH* gene polymorphism.

INSULIN-LIKE GROWTH FACTORS AND THEIR RECEPTOR (*IGF1*, *IGF2*, *IGF1R*) GENES

Growth as a biological phenomenon is controlled by a complex of endo-, para and autocrine control mechanisms. The insulin growth factor (IGF) complex plays a key role in growth regulation together with insulin, thyroid hormones, sex steroids, and the growth hormone. The IGF system consists of two insulin-like growth factors (IGF-I and IGF-II), two receptors, and six binding proteins (IGFBP-1 to -6). IGFs are growth-promoting peptides, which are structurally homologous with insulin and also their biological effects are similar to those of insulin. Insulin is synthesised exclusively in the pancreatic islets of Langerhans, while IGFs are synthesized in tissues throughout the body [Nedbel *et al.*, 2000].

The effect of growth hormone on growth, myogenesis and some metabolic pathways is mediated by IGF-I and IGF-II. This has inclined researchers to evaluate the influence of *IGF-I* and *IGF-II* genes polymorphism on carcass traits. QTLs for average daily gain and back fat thickness, measured at the tenth rib, were mapped on chromosome 5 in the region of *IGF-I* gene localization. Moreover, a significant association has been described between variant of microsatellite sequence localized in the 5' flanking region of *IGF-I* gene and a value of both performance traits [Casas-Carillo *et al.*, 1997]. Very promising results were obtained by Jeon *et al.* [1999] and Nezer *et al.* [1999] concerning the association between performance traits and *IGF-II* genotype. They evidenced that particularly carcass meatiness depended on *IGF-II* genotype, explaining about 20% of the total phenotypic variance of this trait. Usefulness of *IGF-II* gene variants as markers in a mass-selection is limited since the only gene variant expressed in the offspring is that inherited from sire (paternal imprinting).

Our studies have been performed on a population of 322 pigs obtained from mating of sows (Polish Landrace \times Polish Large White) with boars of different commercial breeds (Polish Landrace, Polish Large White, Duroc, Pietrain). The polymorphism of microsatellite sequences localized in *IGF-II* and *IGF-I* genes, described by Kirkpatrick [1992] and Jeon *et al.* [1999], respectively, as well as a single nucleotide polymorphism (SNP) of *IGF1R* gene identified by Kopečný *et al.* [2002] have been defined in the animals mentioned above. The only significant association between gene polymorphism and performance traits was that between microsatellite sequence variant in *IGF-II* gene and fat thickness measured over the shoulder and over the loin [Pierzchała *et al.*, 2004b] (Table 1). The lack of significant influence of *IGF1R* gene polymorphism on carcass traits could be caused by a low frequency of one of the alleles. However, the frequency of *IGF1R* alleles determined in our study was similar to that presented by Kopečný *et al.* [2002]. Thus, it could be assumed that selection programs were more conducive for keeping one of the *IGF1R* allele. One may suppose, that another gene variant was unbeneficial for traits being improved through selection

and due to this it was eliminated. Moreover, it could be concluded, that the polymorphism of *IGF1R* gene, discovered by Kopečný *et al.* [2002], may appear as useless in analysis of its effect on performance traits in pig populations due to monomorphism of this marker.

The *IGF-II* gene has been described in several studies [Nezer *et al.*, 1999; Jeon *et al.*, 1999] as a candidate gene for meat efficiency in pigs. The IGF-II is a member of the growth factors family and has an effect on development of muscle tissue. In contrast, the corresponding receptor gene *IGF2R* is paternally imprinted and expressed when transmitted by the mother. In pigs, the *IGF-II* gene, localized on chromosome 2 (*SSC2*), appears maternally imprinted and expressed only *via* the sire [Nezer *et al.*, 1999]. This gene was marked as a candidate gene for muscle mass (skeletal and cardiac) and fat deposition.

LEPTIN (*LEP*) AND ITS RECEPTORS (*LEPR*) GENES

The *LEP* is 16-k-Da hormonal protein product of the obese gene that acts on central and peripheral tissues to modulate appetite and energy metabolism [Fruhbeck *et al.*, 1998]. It is predominantly secreted by the adipocytes. However, The *LEP* receptors (*LEPR*) are the product of the diabetes gene, and mainly associated with back-fat thickness (BFT) and intramuscular fat content in an F_2 crossed between Iberian and Landrace breeds [Ovilo *et al.*, 2002]. These physiological properties of *LEP* and *LEPR* support as a strong candidate gene for carcass fat content and meat mass. The fat content of carcass is an important polygenic (QTL) trait in pig breeding practices. Research studies indicate that both *LEP* and *LEPR* play an essential role in food intake and energy balance. However, there are several other genes which may play a key role in the regulation of fatness traits in pigs, for example, heart fatty acid binding protein (H-FABP), adipocyte fatty acid binding protein (A-FABP), adipose differentiation related protein (ADFP), CCAAT/enhancer binding protein α, β, γ (C/EBP α, β, γ), cyclic AMP response element binding protein (CREB) and peroxisome proliferator activated receptor γ (PPAR γ) [for detailed review: Świtoński *et al.*, 2003].

In general, the pork industry is interested in the production of lean pork and with the elevated fatness. In this context, *LEP* action, mediated through its specific receptors, plays a vital role in the regulation of fatness *via* feed intake, energy expenditure and whole body energy balance in pigs. The *LEP* and *LEPR* genes are assigned on pig chromosome 18q13-21 and 6q33-35, respectively [Neuenschwander *et al.*, 1996; Ernst *et al.*, 1997].

MYOGENIC REGULATORY FACTORS (*MRF*) GENE FAMILY

Meat quality is a complex trait affected by genetic factors relating back to the prenatal formation of muscle tissue (myogenesis), *i.e.* determination of precursor cells (myoblasts), proliferation (cell division), and differentiation into multinucleated myofibers. This process is an exclusive prenatal event taking place twice, *i.e.* primary and secondary muscle fiber formation, together referred to as myogenesis. Postnatal growth of muscle tissue is characterised by hypertrophic growth of myofibers without the formation of new myofibers.

Myogenesis and postnatal muscle tissue growth are regulated by the myogenic regulatory factors (*MRF*) gene family.

The *MRF* gene family consists of four structurally related transcription factors - myogenin, MyoD1, MRF-4, MYF-5 and MYF-6 – regulate both skeleton muscle fiber development and postnatal hypertrophic growth [TePas & Soumillion, 2001]. The *MyoD* and *MYF-5* genes regulate proliferation of myoblasts and satellite cells (postnatal type) by having the ability to fuse with existing myofibers, but lacking the ability to form new myofibers. Myogenin is expressed during fusion of cells to form multinucleated myofibers. The role of MYF-6 has been mainly described as maintaining the muscle tissue. The *MRF* gene family is considered as a strong candidate gene for skeleton muscle mass, *i.e.* meat mass. The candidate gene approach generally involves first to recognize the gene of a trait on the basis of physiological function, then to study the genomic variation of that gene to detect the ultimate causal mutation leading to modified protein with a single changed activity or a slight change in the level of expression of an unmodified protein. Finally, a candidate gene for the given trait can be validated by means of an association study. In general, an association study involves investigation of resource families with a large population (for example full-sib families) by genotyping of the polymorphic site at candidate gene.

PROTEIN KINASE ADENOSINE MONOPHOSPHATE-ACTIVATED γ 3-SUBUNIT (*PRKAG3*) GENE

Mammalian *adenosine monophosphate (AMP) – activated protein kinase (AMPK)* plays a key role in regulating energy homeostasis in eukaryotes [Hardie *et al.*, 1998]. It consists of a catalytic subunit (α) and two regulatory subunits (β and γ). Two isoforms have been identified for both the α - and β -subunit as well as the third isoforms reported for the γ -subunit in several mammals [Milan *et al.*, 2000]. The γ_3 -peptide, encoded by the *PRKAG3* gene, is one of three options for the γ regulatory subunit of AMPK. When eukaryotic cells are subjected to environmental or nutritional stress factors and the AMP/ATP ratio rises significantly, then the “AMPK cascade” is induced, initiating measures to conserve energy and to induce the ATP synthetic pathways [Hardie *et al.*, 1998].

The identification of quantitative trait loci (QTL) for meat quality traits in the region of the *PRKAG3* gene in an RN^+ resource population [Malek *et al.*, 2001] suggested that new allelic variation in this gene may be responsible for the observed effects. Several studies have shown the presence of new economically important alleles of the *PRKAG3* gene affecting glycogen content of muscle and in general the meat quality traits of pigs that include ultimate pH and color measures and that are correlated with water-holding capacity, drip loss, tenderness, and cooking loss [Seller, 1998; Ciobanu *et al.*, 2001].

The *PRKAG3* gene encodes a muscle-specific isoform of the regulatory gamma-subunit of *adenosine monophosphate-activated protein kinase*, which plays a key role in regulating energy homeostasis in eukaryotes. It is well known that mutations in the *PRKAG3* gene affect high glycogen content in the porcine skeletal muscle and, consequently, meat quality. Several QTLs affecting muscle glycogen content and related traits were mapped to pig chromosome 15. Based on

this QTL locations, Ciobanu *et al.* [2001] demonstrated the causal mutation in the *PRKAG3* gene to strongly affect a low glycogen content in pig skeleton muscle, thus proved it to be a potential candidate gene for improved meat quality. In a previous study, Milan *et al.* [2000], has also confirmed that a non-conserved substitution in the *PRKAG3* gene is accounted for large differences in meat quality and processing yield in the Hampshire pig breed [Monin & Sellier 1985, LeRoy *et al.*, 1990]. This substitution (R200Q: a dominant mutation denoted as RN⁻) in the *PRKAG3* gene caused a 70% increase in glycogen in muscle in RN⁻ homozygous and heterozygous animals that result in the observed lower muscle pH 24 h after slaughter, in reduced water holding capacity in the muscle and in the much lower yield of a cured cooked ham product. Fontanesi *et al.* [2003] showed that a high value of glycolytic potential was not due to the presence of the RN⁻ allele (200Q) in the tested commercial pigs. However, their data confirmed the effect of other mutations at the *PRKAG3* locus (T30N and G52S) on meat quality. Moreover, they observed that muscle glycogen content was significantly dependent on *PGAM2* (*phosphoglycerate mutase 2*) genotype.

MELANOCORTIN RECEPTOR (*MCR*) GENE FAMILY

The melanocortin-4 receptor (*MC4R*), a G protein-coupled seven-transmembrane receptor, which is expressed in the brain, plays an important role in the control of mammalian energy homeostasis and is involved in food intake and body weight regulations, and several mutations within these genes have been significantly associated with fat deposition phenotypes in humans [Lubrano-Berthelier *et al.*, 2003]. Several studies [Kim *et al.*, 2000a; Hernández-Sánchez *et al.*, 2003; Kim *et al.*, 2004a,] revealed that causative and non-causative gene variants of porcine *MC4R* have been associated with obesity-related phenotypes. The study of Kim *et al.* [2004a], concluded that a single missense mutation (Asp298Asn) of aspartic acid (Asp) to asparagine (Asn) in *MC4R* gene decreased cAMP content and *MC4R* signaling, but with no difference in the ligand binding was associated with growth and feed intake traits in domestic pigs. Recently, a similar study has been conducted by Stachowiak *et al.* [2006], in Polish pig breeds. Their preliminary results concluded that the *A* allele was correlated with higher test daily gains and lower levels of intramuscular fat in Polish Landrace (PL), and increased levels of intramuscular fat in Polish Large White (PLW). No evidence of an effect of this polymorphism on daily food intake, back-fat thickness or abdominal fat was observed [Stachowiak *et al.*, 2006].

While, Hernández-Sánchez *et al.* [2003] investigated the population-wide associations between loci due to linkage disequilibrium to utilize for the high-resolution fine mapping of quantitative trait loci (QTL). The study concluded that the transmission-disequilibrium test (TDT) is a robust test for detecting QTL and the results supported the previously reported results; *i.e.* the studied polymorphism is either causal or in very strong linkage disequilibrium with the causal mutation, and provided no evidence for spurious association. In support to melanocortin receptor, the phenotypic association studies were performed by comparative analysis on some other obesity-related candidate genes, for example, porcine agouti signaling protein (*ASIP*), agouti related protein (*AGRP*), High-

mobility Group A (*HMGA*) family genes and Peroxisome proliferator-activated receptor γ (*PPAR-\gamma*). The study revealed that the investigated obesity-related candidate genes in the pig are not only important for development of marker-assisted selection on growth and fat deposition traits in the pig but also contribute to the understanding of their genetic roles in the development of human obesity [Kim *et al.*, 2004b].

In general, *ASIP* and *AGRP* have been demonstrated to play an important role as an inhibitory effects on central melanocortin receptors [Sutter *et al.*, 1997]. The porcine *ASIP* and *AGRP* were previously mapped on *Sus scrofa* chromosome 17 (SSC 17) [Kim *et al.*, 2000b] and SSC 6 [Kim & Rothschild, 2003]. However, the *HMGA* family genes may exert critical roles in adipocytic cell growth and differentiation. The *HMGA1* and *HMGA2* genes were previously mapped to human chromosomes 6p23-p21 and 12q15, respectively [Fedele *et al.*, 2001], and their probable locations on pig chromosomes were assumed to be 7 and 5, respectively, based on the comparative map between humans and pigs. While, the *PPAR-\gamma* is expressed mainly in adipose tissue and influences the storage of fatty acids [Latruffe & Vamecq, 1997].

DISCUSSION

Functional candidate genes related especially to myogenesis for body growth rate and carcass composition traits towards their functional role for the genetic improvement of meat quality and efficiency of the pork production were comprehensively reviewed in this paper. According to the candidate gene approach, studies on association between genotype at particular locus out of those controlling polymorphism of several growth factors and their receptors on one side and performance traits in pigs on the other showed that it was difficult to find any universal causal gene variant among the functional candidate genes for muscle and body growth as well as carcass yield towards their useful implementation in marker assisted selection (MAS) program. Not all significant relationships identified in some breeds and crosses were confirmed in the others. Most probably, gene variants of significant unprofitable effect on traits were eliminated through selection based on phenotype (for instance fat thickness). There are known variants of some genes conditioning muscle hypertrophy being disadvantageous for functionality of the organism, however they might be considered as beneficial for breeders. Stress sensitivity gene in pigs (mutated gene *RYR1*) or mutated myostatin gene in Blue Belgian cattle conditioning muscle hyperplasia are a good examples of such a relation. Next, the *IGF-II* gene mutation resulting in a high meat content of carcass cannot be used in a mass-selection because of specific model of expression of gene variants inherited from parents. Apart from the most popular candidate gene (*RYR1* in case of pig and double muscling or muscular hypertrophy in cattle) for the genetic improvement meat quality, recent research studies are now revealing many other functional candidate genes like *GH*, *GHR*, *GHRH*, *GHRHR*, *IGF*, *IGF-I*, *IGF-II*, *IGF-IR*, *PIT-1*, *PRKAG3*, *LEP*, *LEPR*, *MCR* and *MRF* genes.

CONCLUSIONS

Summarizing a current knowledge concerning polymorphism of genes encoding growth factors and their receptors

and its usefulness in selection of pigs one may accept that these investigations showed:

1. a range of usefulness of these functional candidate genes in mass-selection;
2. the necessity of evaluating the association between some gene variants and performance traits in commercial breeds and crosses;
3. the necessity of identifying new causal mutations in regulatory regions of genes, most probably affecting transcriptional activity of genes leading to hormone concentration in a tissue or blood plasma. This relation could also affect a level of particular performance trait.

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ZASTOSOWANIE OSIĄGNIĘĆ WSPÓŁCZESNEJ GENETYKI DO POPRAWY JAKOŚCI MIĘSA WIEPRZOWEGO – ARTYKUŁ PRZEGLĄDOWY

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Zgodnie z informacjami z baz danych FAO [2002], około 40% mięsa spożywanego rocznie na świecie stanowi mięso wieprzowe. Spożycie tego mięsa rośnie wraz ze wzrostem populacji ludzkiej. W ostatnim dziesięcioleciu wykorzystanie nowoczesnych technik badawczych w kierunku poprawy cech ważnych z ekonomicznego punktu widzenia, takich jak tempo wzrostu z uwzględnieniem rozwoju tkanki mięśniowej oraz procesów metabolicznych przebiegających w tkance tłuszczowej, wydatnie przyczyniło się do poprawy jakości tuszy, jakości mięsa a tym samym efektywności produkcji wieprzowiny. Stosowanie nowoczesnych technologii opartych na badaniach genomiki funkcjonalnej daje szerokie możliwości poznawcze, ale ich zastosowanie w produkcji świń wymaga dokładnej weryfikacji wyników uzyskanych w badaniach naukowych. Poznanie sekwencji genomu świni stwarza możliwość identyfikacji nowych genów kandydujących, oraz regionów regulujących ich ekspresję. Obiecujące są badania, przedmiotem których jest regulacja ekspresji genów związanych z procesem miogenezy. Stąd celem tej pracy jest przegląd i ocena genów kandydujących dla cech użytkowych, które wpływają na wzrost i rozwój tkanki mięśniowej, takich jak: hormon wzrostu (*GH*), receptor hormonu wzrostu (*GHR*), czynnik uwalniania hormonu wzrostu (*GHRHR*), insulinopodobne czynniki wzrostu (*IGFI* i *IGFII*), receptor insulinopodobnego czynnika wzrostu (*IGFIR*), przysadkowy czynnik transkrypcyjny (*PIT-1*), leptyna (*LEP*), receptor leptyny (*LEPR*), rodzina czynników miogennych (*MRF*), podjednostka $\gamma 3$ kinazy białkowej aktywowanej przez adenozynomonofosforan (*PRKAG3*) oraz rodzina genów receptorów melanokortyny (*MCR*).

