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CANDIDATE GENES FOR SLAUGHTER TRAITS IN PIGS

KANDIDATNI GENI ZA KLAONIČKA SVOJSTVA KOD SVINJA

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SUMMARY

Rapid development of DNA research techniques in the last few decades has enabled identification of genes that underlie genetic variation of production traits observed in livestock species. Identification of these genes is expected to allow more efficient selection with employment of genetic markers and to yield more accurate insight into the physiology of the corresponding traits. Majority of production traits are polygenic and the first step in the determination of their genetic background is in searching for so called candidate genes with an impact on a defined trait.

Production traits in pigs, like growth and carcass characteristics, play an important role in pig breeding and selection. Like many other economically important traits in farm animals they are determined by an unknown number of genes together with environmental factors. Meat quality assessment is based on measuring some phenotypic traits like intramuscular fat content, pH-value, electric conductivity, drip loss and color. The study of candidate genes, in connection with phenotypic effects, is an important tool to identify genes to be used in marker-assisted selection programs.

This work is a review of some candidate genes for which it has been established that they have an important impact on carcass traits in pigs, like for example growth hormone gene complex, PPARGC1, pituitary-specific transcription factor, melanocortin receptor and myogenin.

Key words: pigs, candidate genes, carcass traits

INTRODUCTION

The pig was one of the first animals to be domesticated over 7000 years ago and pork is the major red meat consumed worldwide. Coordinated efforts to better understand the pig genome were initiated in the early 1990s with the development of the international PiGMaP gene mapping project. There were two significant linkage maps published by the mid-1990s of which the largest contained over 1,200 microsatellite markers (Archibald et al., 1995; Rohrer et al., 1996). Since that time, growth of the linkage map has slowed though new gene markers such as microsatellites, amplified fragment length polymorphism (AFLPs), and single nucleotide

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polymorphisms (SNPs) have been continuously identified and mapped.

One of the hot interests of current pig quantitative genetics is systematically exploring an exact genetic architecture of the number, distribution and interaction of loci affecting the variations of biomedically, economically, and evolutionarily important complex and quantitative traits. Advances in molecular genetics have opened opportunities to enhance strategies for genetic improvement of pigs by directly selecting on genes or chromosomal regions that harbour genes that affect traits of interest.

There are two main approaches for genetic improvement of farm animals. The first one is searching for markers, associated with quantitative traits and mapping of quantitative trait loci (QTL) over a genome. The ability to dissect genetically quantitative traits has been improved by the development of detailed linkage maps based on DNA markers. With these the segregation of individual chromosome segments can be traced in appropriate pedigrees (Andersson et al., 1994). Initially many QTL experiments were undertaken by using linkage maps to help determine regions underlying traits of importance for the pig industry. These early QTL scans used families developed by generally crossing European Wild Boar with a commercial breed or crossing the exotic Chinese Meishan breed with a commercial breed.

Gene mapping and comparative genomic studies in the pig have been conducted for analysis of the QTL with economic importance and several chromosomal regions have been found to be important for pig growth, fat deposition, and carcass traits (Bidanel and Rotschild, 2002).

Although breed crosses are very powerful to detect QTL, a problem with the breed-cross genome scan approach is that the markers that are found to be associated with the trait in these crosses may actually be quite some distance from the gene that causes the effect. In addition, these approaches detect genes that differ between the breeds that are used in the cross and these genes may not show variation within a breed, which is required for within-breed selection. Both these factors limit the direct utility of results from breed-cross studies for within-breed selection (Rothschild et al., 2007).

More recently researchers have used two commercial breeds for F2 families or large commercial synthetic lines or breeds for candidate gene studies and large scale SNP association analyses. The candidate gene approach utilizes knowledge from species that are rich in genome information (e.g., human, mouse), effects of mutations in other species, previously identified QTL regions, and/or knowledge of the physiological basis of traits to identify genes that are thought to play a role in the physiology of the trait. Following mapping and identification of polymorphisms within the gene, the association of genotype at the candidate gene with phenotype can be estimated in a closed pig breeding population. In contrast to the breed-cross genome scan approach, the candidate gene approach identifies markers that are at or close to the causative gene and that segregate within the breeds. These markers can, therefore, be more directly used for within-breed selection. Extensively examined meat quality genes have been reported and genetic markers identified within these genes now permit genetic testing and therefore have allowed producers to remove the alleles deleterious to meat quality (Rothschild, 2003).

To date, these techniques for finding genes and QTL, in particular the candidate gene approach, have resulted in the discovery of several genes or markers that are used in the industry. Few examples are the ryanodine receptor gene (halothane gene) for meat quality, growth hormone (GH) and growth hormone releasing hormone (GHRH) for growth, development and various metabolic activities regulation, melanocortin 4 receptor (MC4R) and pituitary-specific transcription factor (PIT1) for lean meat content and backfat thickness, PPARGC1 for adipocyte differentiation, calpastatin (CAST) for meat tenderness and myogenin for muscle growth and differentiation (Dekkers and Rothschild, 2007).

OVERVIEW OF CANDIDATE GENES FOR CARCASS TRAITS IN PIGS

Ryanodine receptor mutation associated with MHS

Swine malignant hyperthermia syndrome (MHS) is a pharmacogenetic disease that affects calcium

regulation in muscle and results in sudden death and/or in PSE meat. In living animals, MHS can be induced by halothane anaesthesia, and this challenge has been used for many years as a test to detect the homozygous carriers (nn) (Webb and Jordan, 1978). Because susceptibility to halothane in pigs is controlled by a recessive gene *(Hal)* exhibiting incomplete penetrance, heterozygous animals (Nn) do not show phenotypic signs of MHS.

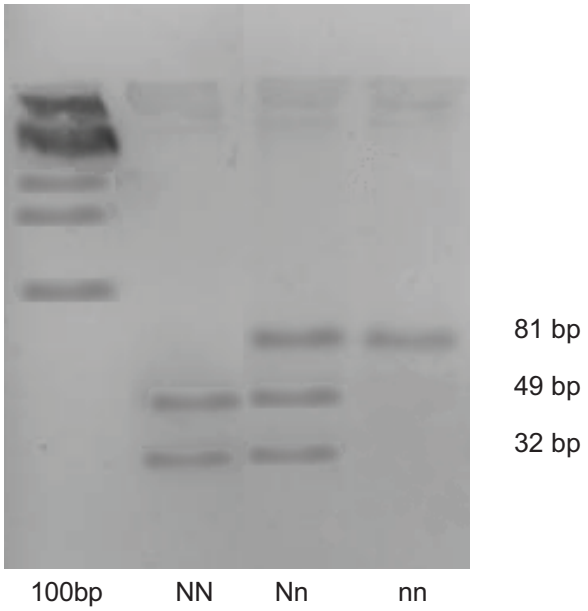

Figure 1. PCR produces specific ryanodine receptor 81-bp DNA fragment. Digestion by *Hhal* yields two DNA fragments of 49 and 32 bp for the C allele (NN) and an intact 81-bp fragment for the T allele (nn). Heterozygous products (Nn) yield all three fragments

Slika 1. PCR proizvodi specifični DNA fragment ryanodin receptora dužine 81-bp. Restrikcija s *Hhal* rezultira fragmentima od 49 i 32 bp za C alel (NN) i jednim fragmentom od 81 bp za T alel (nn). Heterozigorni product (Nn) prikazuje sva tri fragmenta.

Growth hormone (GH) and growth hormone releasing hormone (GHRH)

A C to T transition at nucleotide 1843 of the calcium release channel, also called the ryanodine receptor gene (ryr-1 locus), was associated with MHS susceptibility (Fujii et al., 1991) and a DNA-based test using a polymerase chain reaction (PCR) amplification of the target region coupled with a restriction endonuclease assay was proposed. The occurrence of the mutation was found to be consistent with genotypic results obtained using halothane, progeny testing, and *Phi/Pgd* haplotype analysis of 338 Landrace pigs. It was concluded that C/C, C/T, and T/T allelic pairs were associated to the halothane genotypes NN, Nn, and nn (Houde et al., 1993).

Selection for increased growth rate is one of the most important tools used in pig breeding. The choice of animals for mating determines both growth rates during fattening and final carcass quality in their progeny. Genes directly involved in regulating the expression of growth factors may be interesting as candidate genes for growth rate and carcass quality traits of pigs.

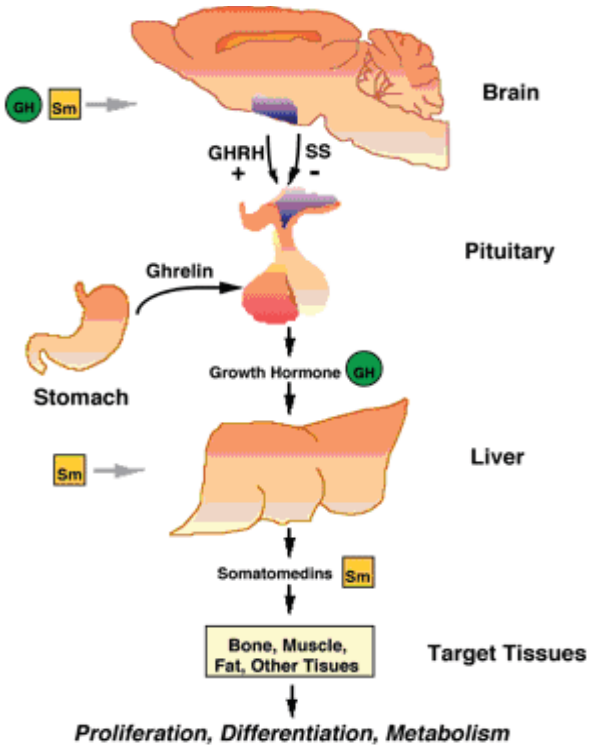

Figure 2. The growth hormone axis

Slika 2. Djelovanje hormona rasta

The GHRH is an endogenous stimulator of somatotropin secretion. It is released by hypo-

thalamus and stimulates the proliferation of pituitary somatotroph cells during their development regulating production and secretion of GH. GH is a peptide hormone with about 190 residues which regulates growth, development and various metabolic activities.

The *Alu*I polymorphism in the third exon of *GHRH* gene was found to be significantly associated with fat thickness and meat content of carcass in different breeds of pigs (Pierzchała M. et al., 2003) and with average daily gain and fat thickness (Franco MM et al., 2005).

Although most amino acids of the GH protein are conserved, there are still many single nucleotide polymorphisms which are reported in the traits of growth, lean rate and milk production. The *GH Dde*I polymorphisms are associated with fat thickness (P = 0.0326) and average daily gain (P = 0.0127) (Franco et al., 2005). Different *Dde*I, *Nar*I and *BsmN*I polymorphisms have been found in the coding sequence of *GH* in different breeds.

Melanocortine 4 receptor (MC4R)

The melanocortin 4 receptor is expressed in virtually all brain regions of mammals and plays an important role in energy homeostasis. Polymorphisms in this gene may thus be related to growth and obesity. In pigs, a non-synonymous polymorphic site was described (Asp298Asn) and demonstrated to affect cAMP production and to alter adenylyl cyclase signalling. Association studies revealed significant linkage of this mutation with production trait in pigs (Kim et al. 2000; Hernandez-Sanchez et al. 2003; Jokubka et al., 2006). This polymorphism was shown to be significantly associated with feed intake, fatness and growth traits in different breeds of pigs. However, the effects were not observed in all investigated lines. The polymorphism is easily genotyped by PCR and subsequent restriction with *Taq*I restriction endonuclease (Jokubka et al., 2006).

Pituitary-specific transcription factor (PIT1)

Pituitary transcription factor (PIT1) has been shown to be a positive regulatory factor of growth hormone, prolactin, and thyrotrophin-β-subunit in the mammalian pituitary. Therefore, the gene encoding PIT1 has been chosen as a candidate gene for growth and carcass traits in pigs.

A number of studies have shown that the PIT1 gene is associated with variation in birth weight (Yu et al., 1996) and weaning weight, average daily gain and backfat in pigs (Yu et al., 1995; Stančekova et al., 1999). However, effects of PIT1 *Rsa*I PCR-RFLP on performance traits in two F2 populations derived from crossing the European Wild Boar, Pietrain and Meishan pigs did not reach genome-wide significance thresholds (p = 0,0000116) (Brunsch et al., 2002).

Calpastatin (CAST)

The rate and extent of skeletal muscle growth depends mainly on three factors: rate of muscle protein synthesis, rate of muscle protein degradation, and the number and size of skeletal muscle cells. Recent studies have shown that calpain activity is required for myoblast fusion (Barnoy, 1997) and cell proliferation in addition to cell growth (Mellegren, 1997). The calpain system may also affect the number of skeletal muscle cells in domestic animals by altering rate of myoblast proliferation and modulating myoblast fusion. A number of studies have shown that the calpain system is also important in normal skeletal muscle growth. Increased rate of skeletal muscle growth can result from a decreased rate of muscle protein degradation, and this is associated with a decrease in activity of the calpain system, due principally to a large increase in calpastatin activity (Goll et al., 1998). These observations suggest that genes coding for calpains and calpastatin may be considered as candidate genes for lean content of carcass in pigs.

By investigating the impact of CAST *Hin*fI, *Rsa*I and *Msp*I genotypes on carcass traits in different pig breeds it was shown that CAST/*Hin*fI genotype had no impact on carcass traits, whereas CAST/*Rsa*I and CAST/*Msp*I affected some of the meat and fat deposition traits in pigs (Kurił et al., 2003). By analysing meat quality traits influenced by CAST/*Msp*I genotype, it was observed that animals with BB genotype at this locus were characterized by the most profitable values of all analysed traits (Krzęcio et al., 2005).

Peroxisome proliferator-activated receptor-gamma coactivator-1 (PPARGC1)

Fat deposition and its body distribution in pigs is economically important for production of pigs, low in back fat but having intramuscular fat, which contributes to the improvement of sensory traits of meat. Recent research has focused on the identification of candidate genes for predicting meat quality and muscle tissue development. The peroxisome proliferator-activated receptor-gamma coactivator-1 (PPARGC1) is a transcriptional coactivator of many nuclear hormone receptors including peroxisome proliferator-activated receptor-gamma, which has been shown to be involved in lipid metabolism and play the central regulatory role in adipocyte differentiation (Rosen and Spiegelman, 2000). Because of its role in body composition and fat distribution PPARGC1 is a candidate gene for fatness and leanness.

The complete coding sequence of the porcine PPARGC1 gene including a single nucleotide polymorphism in exon 8 was recently determined. A T/A substitution at nucleotide position 1378 results in an amino acid substitution (Cys → Ser) at position 430 in the amino acid sequence.

Differences of allele distribution between Chinese and Western pig breeds were described (Kunej et al., 2005). T (Cys) allele was present in all 13 analysed pig breeds. However, A (Ser) allele was present only in Western pig breeds, and in one Chinese (Taoyuan) breed.

RECENT STATUS OF PIG GENOMICS IN CROATIA

Among all known candidate genes for carcass traits in pigs, only genotyping for ryanodine receptor mutation associated with MHS is used in pig selection and breeding in Croatia. In the last decade, all boars used for artificial insemination have been genotyped for the C to T transition at nucleotide 1843 of the calcium release channel, also called the ryanodine receptor gene (ryr-1) locus. Genotyping is carried out in The Centre for livestock improvement in Križevci with a DNA-based test using a PCR amplification of the target region coupled with a restriction endonuclease assay.

At the Agricultural Faculty in Osijek, researches on candidate genes started few years ago in collaboration with the Zootechnical department of the Biotechnical Faculty in Ljubljana. The aim of the study was to determine genetic status of ryr-1 and PPARGC1 genes in two Croatian autochthonous pig breeds, Black Slavonian and Turopolje pig breeds. Results showed that in both breeds only C allele was present on ryr-1 locus, thus both breeds are MHS resistant. Regarding PPARGC1 gene, the A and T alleles were represented in equal amounts (frequency close to 50%) in both breeds (Margeta et al., 2006).

Researches on candidate genes in pigs continued in the scope of the Postdoctoral project, financed by the National Foundation for Science, Higher Education and Technological Development of the Republic of Croatia. 70 pigs (multibreed crosses) were included in the study. Pigs were slaughtered and carcass traits like carcass weight, backfat thickness, muscle content, carcass length, ham length, pH, electric conductivity, water-holding capacity, drip loss and color were measured. Blood samples were taken for DNA extraction and all animals were genotyped on 13 polymorphic sites in seven (ryr-1, GH, GHRH, myogenin, MC4R, PIT1, CAST) candidate genes for carcass traits. Majority of genotyped sites showed high degree of polymorphism and were further included into statistical analysis to determine possible impact of candidate gene genotype on measured carcass traits (unpublished results).

CONCLUSION

In expectation of complete pig genome sequence, understanding a complexity of the pig genome for agricultural purposes remains a significant challenge. In the past decade, employment of molecular techniques has enabled large-scale gene and trait identification and mapping and a number of gene tests to improve pork production are in use in the pig industry. Prime examples are the ryanodine receptor gene (halothane gene) for meat quality, the estrogen receptor gene for litter size, and genetic markers for QTL for growth, backfat, litter size and disease on several chromosomes.

Because of the direct impact of candidate gene polymorphisms on most important production and carcass traits in pigs, in the future introduction of

marker assisted selection (MAS) in pig breeding and industry will be also unavoidable in Croatia. This will enable faster progress of pig's genetics and improvement of production, reproductive and carcass traits which are at present still far from those in countries with highly developed pig production.

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SAŽETAK

Brzi razvoj tehnika za istraživanje DNA zadnjih desetljeća omogućio je identifikaciju gena koji su temelj genetske raznolikosti produktivnih svojstava uočenih kod domaćih životinja. Identifikacija ovih gena može povećati uspješnosti selekcije temeljene na genskim markerima i dovesti do točnijeg razumijevanja fiziologije odgovarajućih svojstava. Većina proizvodnih svojstava svinja je određena većim brojem gena i prvi korak u određivanju njihove genske osnove je istraživanje tzv. kandidatnih gena i njihov učinak na određena svojstva.

Proizvodna i klaonička svojstva kod svinja imaju veliku važnost u uzgoju i selekciji. Kao i mnoga druga ekonomski važna svojstva domaćih životinja, i ova svojstva su određena neutvrđenim brojem gena u interakciji s okolišnim čimbenicima. Intenzivna selekcija svinja na visoku mesnatost za posljedicu je imala narušavanje proizvodnih, kao i svojstava kakvoće mišićnog tkiva kod nekih pasmina. Sadržaj intramuskularne masti, profil masnih kiselina, pH-vrijednost, boja mesa, električna provodljivost i sposobnost vezanja vode svojstva su koja u znatnoj mjeri određuju kakvoću mišićnog tkiva svinja. Većina ovih svojstava genetski je uvjetovana, tako da je selekcijom moguće utjecati na njihovo poboljšanje. Proučavanje kandidatnih gena, povezano s fenotipskim učinkom, je značajno oruđe za određivanje gena koji će se koristiti u selekciji.

U ovom radu dan je pregled nekih kandidatnih gena za koje je utvrđeno da imaju značajan utjecaj na klaonička svojstva svinja, kao što su kompleks gena za hormon rasta PPARGC1, hipofizno-specifični transkripcijski faktor, melanokortinski receptor i miogenin.

Ključne riječi: svinje, kandidatni geni, klaonička svojstva