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**Review article**

# Genetics of fat tissue accumulation in pigs: a comparative approach

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**Abstract**. Fatness traits are important in pig production since they influence meat quality and fattening efficiency. On the other hand, excessive fat accumulation in humans has become a serious health problem due to worldwide spread of obesity. Since the pig is also considered as an animal model for numerous human diseases, including obesity and metabolic syndrome, comparative genomic studies may bring new insights into genetics of fatness/obesity. Input of genetic factors into phenotypic variability of these traits is rather high and the heritability coefficient ($h^2$) of these traits oscillates around 0.5. Genome scanning revealed the presence of more than 500 QTLs for fatness in the pig genome. In addition to QTL studies, many candidate gene polymorphisms have been analyzed in terms of their associations with pig fatness, including genes encoding leptin (*LEP*) and its receptor (*LEPR*), insulin-like growth factor 2 (*IGF-2*), fatty acid-binding proteins (*FABP3* and *FABP4*), melanocortin receptor type 4 (*MC4R*), and the *FTO* (fat mass and obesity-associated) gene. Among them, a confirmed effect on pig fatness was found for a well-known polymorphism of the *IGF-2* gene. In humans the strongest association with predisposition to obesity was shown for polymorphism of the *FTO* gene, while in pigs such an association seems to be doubtful. The development of functional genomics has revealed a large number of genes whose expression is associated with fat accumulation and lipid metabolism, so far not studied extensively in terms of the association of their polymorphism with pig fatness. Recently, epigenomic mechanisms, mainly RNA interference, have been considered as a potential source of information on genetic input into the fat accumulation process. The rather limited progress in studies focused on the identification of gene polymorphism related with fatness traits shows that their genetic background is highly complex.

**Keywords**: candidate genes, epigenomics, fatness, genomics, human, mouse, obesity, pig, QTL.

Knowledge on the genetic background of fat tissue accumulation is important in livestock production. Several fatness traits are considered in pig breeding improvement, most frequently backfat thickness (BFT) and intramuscular fat content (IMF). However, some others are also of interest: abdominal fat weight (AFW) or percentage (AFP), and fatty acid composition (FAC). The fatness traits are related to meat quality (mainly IMF and FAC) or fattening efficiency (mainly BFT and AFW). IMF is responsible for marbling, which influences meat tenderness and eating quality (Fortin et al. 2005), while FAC, especially in terms of the polyunsaturated fatty acid (PUFA) ratio, is related to the dietetic value of meat (Wood et al. 2003). An excessive fat accumulation (BFT and AFW) reduces fattening efficiency due to a higher feed conversion rate in case of fat accumulation, when compared with protein accumulation.

Excessive fat accumulation is extensively studied also in humans, due to the worldwide spread of the obese phenotype. Obesity is usually defined by an increased body mass index (BMI). Recently the prevalence of obesity (BMI >30 kg $m^{-2}$) in European populations has reached 28.3% in men and 36.5% in women (Berghöfer et al. 2008). In addition, obesity is considered as an important risk factor for metabolic syndrome (MetS, also known as

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insulin-resistance syndrome). It is also widespread worldwide, and affects up to 25% of the US population (Joy et al. 2008). Both pathologies (obesity and MetS) have a complex background and thus genetic predisposition is intensively studied. In such studies, animal models play an important role, in particular the inbred and selected mouse strains (Pomp et al. 2008). However, also large domestic mammals, including the pig, have recently received attention as useful animal models for human pathologies (Brambilla and Cantafora 2004; Lunney 2007).

Fatness is classified as a quantitative trait with a high contribution of genetic variation. Thus, searching for DNA polymorphism associated with (or responsible for) fat accumulation is reasonable. In this review we present recent progress in these studies. Moreover, usefulness of the comparative genomics approach in searching for functional or associated polymorphisms is discussed.

**Fatness differences between pig breeds and heritability of fatness traits**

For several decades the main objective of pig breeding programs was to increase growth rate and the lean to fat ratio. Selection based on lean growth rate made it possible to decrease BFT by 0.4 mm during each year of selection (Chen et al. 2002). Meat-type modern pig breeds, such as Large White, Landrace, Duroc, Hampshire, Pietrain, and their crosses, are the main sources of pork in developed countries. The meat-type breeds are characterized by fast daily weight gain, especially during the first 8–9 months of life, and the final weight is 200–350 kg. Usually pigs of this type are slaughtered at 100–120 kg of body weight, when their fat content in the carcass is still low. During later development, fat accumulation is faster than muscle growth, which increases the cost of pork production. On the other hand, fat-type pig breeds (Iberian Pig, Mangalica, and Zlotnicka Spotted - a Polish autochthonous breed) grow slowly and reach final weight between 30 kg (miniature pigs) and 200 kg. Although they produce meat of high quality, they are not reared as fast-growing lean meat-type pigs due to their extreme fatness, which makes their breeding more costly.

The ability of some breeds or even single individuals to accumulate large amounts of fat during periods of food shortage was named the *thrifty genotype*. It enabled primitive pig breeds, such as Iberian or Ossabaw, to adapt to repetitive seasonal cycles of fasting and famine. Pigs exhibiting the *thrifty genotype* can be used as animal models of metabolic syndrome (e.g. Ossabaw pigs), but also in the production of traditional pork products, e.g. ham from Iberian Pigs (Dyson et al. 2006; Gonzalez-Anover et al. 2009).

Recently attention in pig breeding programmes is increasingly attracted to improvement of additional pork traits, e.g. organoleptic properties, due to increasing consumer demands. While excessive AFW and BFT are undesirable, IMF is among the factors positively influencing meat sensory traits, such as tenderness, juiciness, and flavour. IMF content is defined as a proportion of total lipids in the muscle tissue, i.e. perimysium, endomysium, triglyceride droplets inside muscle fibres and membrane lipids, but excluding fasciae, crude tendons, and epimysium (Fischer 2005). The suggested optimal range of IMF content to achieve eating satisfaction and meet dietetic requirements is 2.5–3.0% (Fernandez et al. 1999). IMF content depends on the breed as well as muscle type. Examination performed on pigs representing various breeds and their crosses (German Landrace, German Large White, Duroc, Pietrain, etc.) showed that the average IMF content can vary from 1.1% in rectus femoris to 7.0% in semispinalis capitis (Fischer 1994). In some of the most valuable porcine muscles, i.e. longissimus dorsi and semimembranosus, the average IMF content is 1.7% and 2.9%, respectively. These disproportions persist during postnatal development (Fischer 1994; Fischer et al. 2006). Moreover, there are differences in IMF within the same muscle depending on localization. For example, IMF content in the thoracic part (at 5th rib) of longissimus dorsi was higher (1.9%) than in the lumbar part, at the 15th rib (1.3%).

It is important to point out that the correlation between IMF and unfavourable BFT is not very high ($r$ = 0.37) (Hovenier et al. 1992). However, most of modern meat-type pig breeds do not reach the optimal IMF range due to extensive selection towards decreased BFT and increased lean meat content. For example, IMF content in longissimus dorsi in Pietrain, Landrace, and Large White pigs is relatively low and varies from 1.0% to 1.7%, while primitive local breeds (e.g. Mangalica) have much higher IMF levels, up to 7.5% (Table 1). On the other hand, the Duroc breed presents not only an optimal IMF content (2.0–3.3%) in longissimus dorsi, but also a relatively low BFT. This breed, due to its high meat quality, is often used in breeding programs (Florowski et al. 2006a, 2006b).

**Table 1**. Variability of intramuscular fat content (IMF) content of longissimus dorsi in selected pig breeds.

| Breed | IMF (%) | Reference |
|---|---|---|
| Pietrain | 1.1 | Gotz et al. 2001 |
| | 1.3 | Laube et al. 2000 |
| | 1.7 | Florowski et al. 2006b |
| German Landrace | 1.4 | Gotz et al. 2001 |
| Polish Landrace | 1.3 | Florowski et al. 2006b |
| | 1.7 | Orzechowska et al. 2008 |
| Polish Large White | 1.4 | Florowski et al. 2006b |
| | 1.7 | Orzechowska et al. 2008 |
| Duroc | 2.0 | Laube et al. 2000 |
| | 2.9 | Florowski et al. 2006b |
| | 3.3 | Newcom et al. 2004 |
| Zlotnicka Spotted | 3.1 | Florowski et al. 2006a |
| Pulawska | 2.5 | Florowski et al. 2006a |
| | 3.6 | Grzeskowiak et al. 2006 |
| Iberian Pig | 6.0 | Daza et al. 2006 |
| Mangalica | 7.5 | Hollo 2004 |

Since fatness traits have a polygenic background, there are numerous publications presenting estimates of their heritability coefficients ($h^2$). A majority of them concern only the main fatness traits: IMF and BFT. Examinations before 1997, reviewed by Clutter and Brascamp (1998) and Sellier (1998), showed that mean $h^2$ values for IMF and BFT are very similar and oscillate around 0.5. Recent estimations of this coefficient (Table 2) suggest that $h^2$ for IMF is rather below 0.5, while for BFT it is above 0.5. In humans, $h^2$ estimates of BMI are lower, varying between 0.25 and 0.4 (for a review, see Lee 2009).

The above-mentioned results show a substantial impact of genetic factors on phenotypic variability of fat deposition. Thus, searching for quantitative trait loci (QTLs) and functional or associated DNA polymorphisms has become an obvious goal of studies carried out in humans as well as domesticated animal species.

**Table 2**. Recent data on heritability ($h^2$) of intramuscular fat (IMF) and backfat thickness (BFT) in pigs.

| Trait | Breed | $h^2$ | Reference |
|---|---|---|---|
| IMF | Large White | 0.38 | Knapp et al. 1997 |
| | Large White | 0.44 | Larzul et al. 1997 |
| | Large White | 0.35 | Hermesch et al. 2000 |
| | Landrace | 0.67 | Knapp et al. 1997 |
| | Landrace | 0.35 | Hermesch et al. 2000 |
| | Iberian Pig | 0.25 | Fernandez et al. 2003 |
| | Duroc | 0.39 | Suzuki et al. 2005 |
| | Pietrain | 0.42 | Knopp et al. 1997 |
| | Mean | 0.41 | |
| BFT | Landrace | 0.56 | Kuhlers et al. 2001 |
| | Duroc | 0.72 | Suzuki and Nishida, 2006 |
| | Duroc | 0.58 | Kuhlers et al. 2001 |
| | Large White | 0.43 | Nguyen and McPhee, 2005 |
| | Mean | 0.57 | |

## QTL regions for fat tissue accumulation in the pig genome

Numerous QTLs for fatness traits have been recorded as a result of genotyping of 2-generation reference families. The families have usually been developed by crossing wild boar or the Meishan breed with a commercial breed. The first QTL for fat deposition in the pig was localized on chromosome 4 (SSC4) between the *ATP1B1* and *S0001* loci (Andersson et al. 1994). This QTL was indicated as accounting for a substantial part of the variation in fatness. Although the discovery of the QTL on SSC4 was confirmed by many independent studies in various populations, to date the causal mutation has not been identified or even strongly suggested. This region, named as *FAT1*, still remains elusive (Marklund et al. 1999; Mercadé et al. 2006; Estellé et al. 2006). In 2006 the crucial region of *FAT1* was narrowed down to a 3.3-cM interval, between the *RXRG* and *SDHC* loci (Berg et al. 2006). Recent cytogenetic mapping of 8 genes of the *FABP* gene family showed that a cluster of 3 genes, including a candidate gene for pig fatness (*FABP3*), are tightly linked and located outside this narrowed interval (Szczerbal et al. 2007).

The comparative analysis (Figure 1) revealed that regions orthologous to the porcine *FAT1* locus were found in both human (chromosome 1; HSA1) and murine (chromosome 1; MMU1) genomes (Berg et al. 2006). This finding is supported by the identification of QTLs for obesity in these regions. In HSA1q23 there is strong evidence for QTLs contributing to the obese phenotype in American, Asian, and Pima Indian populations (Mutch and Clement 2006). In the *FAT1* orthologous region of MMU1, a QTL for obesity was detected and 3 candidate genes were selected for expression study: *Fmo1*, *Fmo3,* and *Apoa2* (Brown et al. 2005). The analysis showed that among these genes only *Fmo1*, encoding flavin-containing monooxygenase 1, presented a significant difference in terms of transcript level, and was 4.8-fold down-regulated in the adipose tissue of TH mice with type 1 diabetes, as compared to healthy controls.

Currently, there is ample evidence for QTLs for fat tissue accumulation located on various chromosomes. Beside SSC4, primarily SSC1, SSC6, SSC7, and SSCX have been mentioned as carriers of QTLs

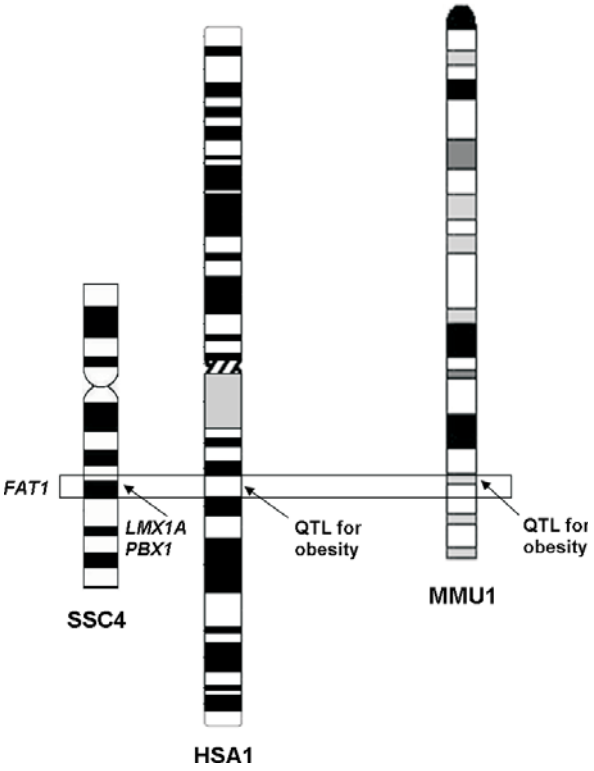


**Figure 1**. Chromosomal localization on human chromosome 1 (HSA1) and mouse chromosome 1 (MMU1) of regions orthologous to the porcine *FAT1* region on chromosome 4 (SSC4). The localization of the QTL for obesity on MMU1 is based on Brown et al. (2005) and Taylor et al. (2001), and for *HSA1* is based on Mutch & Clement (2006). The analysis was performed using MGI Mammalian Orthology (http://www.informatics.jax.org/orthology.shtml) and ENSEMBL database (http://www.ensembl.org). The localization on SSC4 of the most promising candidate genes: LIM homeobox transcription factor 1 alpha (*LMX1A*) and pre-B-cell leukaemia transcription factor (*PBX1*) is shown (Berg et al. 2006).

for fatness traits. Among them, SSC7 seems to contain the highest number of QTLs affecting fat deposition, carcass composition, and growth (Demars et al. 2006). All pig QTL data have been gathered in the pig QTL database (PigQTLdb, http://www.genome.iastate.edu/cgi-bin/QTLdb/SS/index), which is considered as a valuable tool for comparing, confirming, and locating regions of interest in the pursuit of genes responsible for quantitative traits important in pig production. The PigQTLdb contains 5621 QTLs, reported in 237 publications and concerning 546 traits. The number of QTLs for fatness was extended to 911, while the number of QTLs for fat composition reached 224. Among then reported QTLs, more than a half remain suggestive. Top 8 fatness traits in terms of the number of QTLs reported are summarized in Table 3. Among them, the highest number of QTLs was related to BFT and they were identified in almost all pig chromosomes, with the exception of SSC16, SSC17, and SSCY. QTLs for IMF were found on 11 chromosomes (SSC1, SSC2, SSC4, SSC5, SSC6, SSC7, SSC8, SSC9, SSC15, SSC17 and SSCX). However, SSCY was very likely not included in most QTL studies.

**Table 3**. Top 8 fatness traits in terms of number of QTLs reported (http://www.animalgenome.org/QTLdb/pig.html)

| Trait | Significant QTLs | Suggestive QTLs | Total QTLs |
|---|---|---|---|
| Average backfat thickness | 121 | 70 | 191 |
| Backfat at 10th rib | 32 | 35 | 67 |
| Backfat at last rib | 20 | 43 | 63 |
| Intramuscular fat content | 20 | 35 | 55 |
| Backfat at last lumbar | 14 | 22 | 36 |
| Backfat weight | 17 | 15 | 32 |
| Body fat percentage | 22 | 5 | 27 |
| Backfat depth at last rib | 16 | 3 | 19 |

Fat accumulation is strongly correlated with feed intake. Such correlations were reported for several carcass composition traits, including BFT and amount of fat depots (Gilbert et al. 2007). Genome scanning for QTLs for feed consumption and feeding behavior showed their localization close to the known QTLs for meat quality, carcass composition, and growth traits on SSC1, SSC3, SSC7, and SSC9 (Zhang et al. 2009).

Recently, also QTLs for serum lipids (low-density-lipoprotein cholesterol LDL, high-density-lipoprotein cholesterol HDL, total cholesterol, and triglycerides) have been found in the pig. Interestingly, most of them are orthologous to the QTLs previously reported in humans and mice (Gallardo et al. 2008). In a population of Duroc pigs the most significant QTLs for triglycerides and LDL levels at 190 days were located on SSC4 and SSC13, respectively. Other QTLs were found on SSC1, SSC3, SSC6, and SSC12. In another study, genome scanning of the F2 generation of White Duroc Erhualian resource family revealed 1% of genome-wide significant QTLs for LDL, total cholesterol, and triglycerides on SSC2, and for LDL on SSC8 (Chen et al. 2009). Moreover, the fatness traits IMF and FAC determine the dietetic value and taste of pork. A higher percentage of PUFA is associated with deteriorated meat quality due to its softness and excessive oxidation rate. On the other hand, these types of fatty acids are beneficial from the dietary point of view. Thus, an appropriate IMF content and proportion of PUFA are essential for the culinary and dietetic value of pork. Following this, an increasing interest is addressed to FAC. The first QTL for this trait was

mapped to SSC4 by Pérez-Enciso et al. (2000). Its next QTLs were found again on SSC4 and in addition on SSC6, SSC8, SSC10, and SSC12 (Clop et al. 2003). Sanchez et al. (2007) located QTLs for 21 fatty acid composition traits on numerous chromosomes (SSC1, SSC2, SSC4, SSC5, SSC7, SSC8, SSC9, SSC10, SSC11, SSC12, SSC13, SSC14, SSC15, SSC16, and SSC18). As opposed to previous studies, they presented QTLs for FAC of meat, not of backfat. The genetic background of fatty acid composition seems to be a complex issue, since a recent study showed that this trait is partly controlled by epistasis (Uemoto et al. 2009). That study identified 5 pairs of QTLs, located on 4 chromosomes (SSC2, SSC4, SSC5, and SSC16), interacting that way.

## Candidate genes for lipid metabolism – a lesson from studies on the mouse and human obesity

The selection of candidate genes can be based on the function of the encoded proteins in physiological processes governing energy homeostasis. In mammals, fat deposition rate depends on multiple factors related to central nervous system signaling by neuropeptides or the response of peripheral organs (e.g. liver, muscle, and fat tissue). These processes have been reviewed recently by Redinger (2009).

### Mouse model

Present knowledge on obesity genetics has been mainly derived from research on mouse models with spontaneous or targeted single gene mutations as well as polygenic models. Firstly identified genes, whose mutations caused obesity, were those encoding leptin and its receptor. Afterwards, other mutations responsible for the same phenotypic effect were identified in $A^y$ (agouti signaling peptide), *Atrn* (attractin), *Cpe* (carboxypeptidase E) and *Tub* (insulin signaling protein) genes (Brockmann and Bevova 2002). The encoded peptides (leptin, leptin receptor, agouti, and carboxypeptidase E) are components of the central melanocortin system, which play a crucial role in energy homeostasis (Bolze and Klingenspor 2009). Additional genes, whose mutation may cause obesity, were identified by the development of knockout mouse models (Yang et al. 2009). Thereby the role of products of some genes was determined, e.g. mice lacking a functional *Pomc1* gene exhibit obesity and hyperphagia, while inactivation of the *Mc4r* gene results in increased adiposity associated with hyperphagia, hyperinsulinaemia, and hyperglycaemia. Similar effects (increased fat mass) were observed in mice lacking the *Mc3r* gene. The number of single gene mutations that affect energy balance is much bigger. Their role in explanation of the function of a disrupted gene is significant, but such extreme knockout mutations are very rare in human and animal populations. Thus it was assumed that obesity is generally caused by an interaction between environmental factors and an unknown number of genes with small effects.

A common approach in studies of quantitative traits (e.g. obesity), which are determined by many genes and their interaction with environmental factors and gene-gene interactions, is to develop reference families by crossing several mouse strains with various phenotypes and known genotypes. A meta-analysis based on more than 30 publications on mouse cross-breeding experiments, revealed altogether 279 QTLs (162 for body weight and 117 for fat weight) and the major regions were found on mouse chromosomes 1, 2, 7, 11, 15, and 17 (Wuschke et al. 2007). The large number of the identified QTLs reflects the complexity of the genetic background of these traits, specially if gene interactions are considered. There are reports focused on detection of such interactions, and it was found that epistasis is an important source of genetic variance for fat accumulation (for a review, see Warden et al. 2004).

The better understanding of complex diseases, such as obesity, requires the development of new tools. The Collaborative Cross, which is a large panel of recombinant inbred mice strains of the initial cross of 8 inbred strains, is supposed to be such a tool. A complete characterization of the genetic structure of the various recombinant inbred strains and their ability to produce an unlimited number of mice over many generations make this tool very helpful in studies of traits whose variability is affected by numerous factors. It is also necessary to develop statistical models evaluating the impact of genotype and environment on phenotype (The Complex Trait Consortium, 2004).

### Human obesity

Identification of the leptin and leptin receptor gene mutations, causing monogenic obesity in mice, provided an incentive for similar studies in humans. Albeit mutations in these genes are associated with severe obesity in some human patients, it was quite surprising that the contribution of mutations of both genes to monogenic human obesity is very low. There are only 3 reports on causative mutations of the *LEP* gene: frameshift mutation Δ133G (Montague et al. 1997), substitution

R105W (Strobel et al. 1998), and missense mutation N103K (Mazen et al. 2009). These mutations are not widespread. Moreover, Clement et al. (1998) described a case of monogenic obesity as an effect of G/A substitution in exon 16 of the human *LEPR* gene, which caused inactivation of the encoded receptor due to the lack of transmembrane and intracellular domains.

In general, the number of identified gene mutations responsible for monogenic human obesity is small (Rankinen et al. 2006). Among 170 cases described worldwide, the majority was caused by mutations of the type 4 melanocortin receptor gene (*MC4R*), which encodes an important protein of the melanocortin-leptin system of energy homeostasis control (Martinez-Hernandez et al. 2007). First reports documenting the role of *MC4R* gene mutations in the development of monogenic obesity were published by Yeo et al. (1998) and Vaisse et al. (1998). Another gene encoding a receptor belonging to the same family as *MC4R*, whose polymorphisms were reported to cause monogenic obesity, is type 3 melanocortin receptor gene (*MC3R*), which plays an important role in the inhibition of energy storage. Lee et al. (2002) described the novel Ile183Asn mutation of the *MC3R* gene found in an obese Indian girl and her father, suggesting that this substitution may be a previously unknown reason of monogenic obesity. Functional analysis by Tao et al. (2004) indicated that the Ile183Asn mutation at the second intracellular loop of the MC3R protein results in disrupted signal transduction. It can explain the similar phenotypic effect on the monogenic obesity development as the causative mutations of the *MC4R* gene.

Another gene encoding a protein functionally related with melanocortin receptors is the *POMC* (proopiomelanocortin) gene, encoding a precursor of the melanocortins. Krude et al. (1998) and Challis et al. (2002) described several substitutions in the coding region of *POMC*, which are involved in early-onset obesity formation through various molecular mechanisms, i.e. 7013G>T transversion in the third exon resulted in POMC peptide truncation and a complete absence of POMC-derived hormones: ACTH, α-MSH, and β-endorphin. Another mutation, R236G, disrupts the cleavage site between β-MSH and β-endorphin, resulting in the production of mutant MSH/β-endorphin fusion protein, which can bind to MC4R, but its ability to activate the receptor is reduced when compared to natural ligands of the MC4R protein.

Additionally, the group of mutant genes that were found in monogenic human obesity include human proprotein convertase 1 gene (*PCSK1*), encoding an enzyme involved in endoproteolysis of inactive propeptides (i.e. POMC) to release bioactive fragments (Jackson et al. 1997, 2003); genes encoding receptors that play a role in controlling food intake, namely corticotrophin-releasing hormone receptors 1 and 2 (*CRHR1, CRHR2*) (Challis et al. 2004), the G-protein-coupled receptor 24 (*GPR24*) (Gibson et al. 2004), the neurotrophic tyrosine kinase receptor type 2 (*NTRK2*) (Yeo et al. 2004), and the single-minded homolog 1 (*SIM1*) gene, encoding a transcriptional factor expressed mainly in the hypothalamus and potentially involved in energy homeostasis regulation pathways (Holder Jr. et al. 2000; Faivre et al. 2002).

Development of single-nucleotide polymorphism (SNP) microarrays has facilitated application of genome-wide association (GWA) studies. This approach revealed new candidate genes, which may contribute to genetic variance of the obese phenotype (Table 4). The analysis comprised a very large number of patients – over 30 thousands (Willer et al. 2009; Lindgren et al. 2009). Among the listed genes, especially *FTO, MC4R*, and *INSIG2* were reported as associated with predisposition to obesity in several independent studies. The strongest and most repeatable effect was found for the *FTO* gene polymorphism, which explains about 0.1–0.5% of BMI variance. Also the association of common polymorphisms of the *MC4R* gene with obesity was confirmed in different studies. The effect of the insulin-induced gene 2 (*INSIG2*) reported in first GWA studies for body weight trait by Herbert et al. (2006) was confirmed in some of the subsequent reports (for a review, see Hinney et al. 2010).

Genes responsible for human and mouse obesity or associated with polygenic obesity are potential candidate genes that may predispose to fat tissue accumulation also in pigs. However, it should be pointed out that there are important target traits differences in such studies. In humans, the obese phenotype is mainly measured as BMI, while in livestock the traits of interest are BFT, IMF and FAC (but not AFW). One can assume that different genes are involved in the development of different fatness depots and thus selection of putative candidate genes, based on studies of human and mouse obesity, might not be the best approach for pigs.

**Table 4**. An overview of loci associated with human obesity

| Locus name/candidate gene (genes) | Estimated effect on obesity-related traits (% of variability) | Number of subjects | References |
|---|---|---|---|
| *FTO* | 0.34 (BMI) | 32387 | Willer et al. 2009 |
| *MC4R* | 0.10 (BMI) | | |
| *THEM18* | 0.13 (BMI) | | |
| *KCTD15* | 0.01 (BMI) | | |
| *GNPDA2* | 0.13 (BMI) | | |
| *SH2B1* | 0.08 (BMI) | | |
| *MTCH2* | 0.02 (BMI) | | |
| *NEGR1* | 0.03 (BMI) | | |
| *TFAP2B* | 0.05 (WC) | 38580 | Lindgren et al. 2009 |
| *MSRA* | 0.04 (WC) | | |
| *LYPLAL1* | 0.02 (WHR) women only | | |
| *NPC1* | no data | 14186 | Meyre et al. 2009 |
| *MAF* | no data | | |
| *PTER* | no data | | |
| *PRL* | no data | | |
| *INSIG2* | no data | 16969 | Lyon et al. 2007 |
| *SEC16B* | no data | 25344 | Thorleifsson et al. 2009 |
| *ETV5* | no data | | |
| *BDNF* | no data | | |
| *FAIM2, BCDIN3D* | no data | | |
| *NCR3, AIF1,BAT2* | no data | | |

BMI = body mass index, WC = waist circumference, WHR = waist-hip ratio

**Polymorphism of porcine candidate genes for fat accumulation**

Among the numerous candidate genes for pig fatness, a significant group comprises the abovementioned genes, whose mutations cause human obesity. Genome-wide association analyses between gene polymorphisms and fatness traits show that at least 23 genes can be considered as potential candidates (Table 5). Genes that have repeatedly shown association with human obesity are *FTO* (fat mass and obesity associated) and *MC4R*.

The porcine *FTO* gene polymorphism demonstrates only a slight association with fatness traits in pigs. A significant association between g.276T>G polymorphism and IMF was described by Fontanesi et al. (2008) in the Italian White Duroc breed. Also the recent report of Fan et al. (2009a) suggested associations of selected SNPs in this gene and production traits (BFT, marbling, total lipid content, and average daily gain) in the Yorkshire Berkshire cross. However, the authors concluded that the effect of these polymorphisms in pigs seems to be not as spectacular as in humans.

The *MC4R* gene was regarded as a strong positional and functional candidate for porcine fatness, due to the role of the encoded protein in energy balance regulation and chromosomal localization on SSC1, in the centre of the QTL for BFT and meat quality traits. Its first polymorphism associated with daily gain, feed intake, and pig fatness, was reported by Kim et al. (2000), who described the missense substitution c.982A>G (Asp298Asn). The effect of this polymorphism turned out not to be uniform across breeds, especially in terms of fatness traits (Jokubka et al. 2006; Stachowiak et al. 2006; Van den Maagdenberg et al. 2007; Fan et al. 2009a,b). Thus, it was suggested that this SNP might be in linkage disequilibrium with an unknown causal mutation in genetically different breeds (Ibeagha-Awemu et al. 2008).

At present only one polymorphism seems to demonstrate a direct effect on porcine fatness. It is the G3072A substitution in the third intron of the *IGF2* (insulin-like growth factor 2) gene, affecting fat deposition, muscle growth, and heart size (Van Laere et al. 2003; Kolaríková et al. 2003; Estellé et al. 2005; Oczkowicz et al. 2009). Importance of the other genes (Table 5) needs to be verified.

**Variability of expression levels of genes in relation to fat accumulation**

Differences between pig breeds, in terms of fatness, may also be the result of gene expression

**Table 5**. Polymorphism of selected porcine candidate genes for fatness and association studies.

| Gene | Polymorphism | Association study | | Reference |
|---|---|---|---|---|
| | | Breed | Effect of a given genotype/allele | |
| 1 | 2 | 3 | 4 | 5 |
| *ACACA* | c.4899G>A<br>c.5196T>C | Duroc | A4899/C5196 (haplotype)<br>IMF ↓ | Gallardo et al. 2009 |
| *APOE* | c.43+170C>T | LW × L | Allele C<br>BFT (at 10th rib) ↑ | Fan et al. 2009b |
| *CART* | STR<br>(CA)2(CG)n(CA)n<br>(intron 2) | PLW | Allele 253 bp<br>AF ↑ | Stachowiak et al. 2009 |
| *CTSK* | g.15G>A | ID | GG<br>BFT ↓ | Fontanesi et al. 2010 |
| *FABP3* | SNP HinfI (5' UTR) | Duroc | HH genotype<br>IMF ↑ | Gerbens et al. 1999 |
| | | | HH genotype<br>IMF EBF ↓ | Schwab et al. 2009 |
| | g.-158T>G | L990 | Allele G<br>BFT ↑ | Chmurzynska et al. 2007 |
| *FABP4* | STR<br>(CA)n<br>(intron 1) | Duroc | A1A3 (CA22/CA19)<br>IMF ↑ | Gerbens et al. 1998 |
| | g.9T>G | PLW<br>PL | no effect | Chmurzynska et al. 2008 |
| | STR<br>(CA)n<br>(intron 1) | PL | Allele 253 bp<br>BFT (sacrum III) ↑ | Chmurzynska et al. 2004 |
| *GFAT1* | g. 101A>G | LW<br>L | Allele A<br>BFT (corrected) ↑ | Liu et al. 2010 |
| *GNRHR* | c.-854T>G | LW × L | GG<br>BFT (at 10th rib) ↑ | Fan et al. 2009b |
| *IGF-1* | STR<br>(CA)n<br>(intron1) | Landrace | Allele 195 bp<br>BFT ↑ | Estany et al. 2007 |
| *IGF-2* | g.3072G>A | PLW<br>PL | Allele A<br>BFT ↓ | Oczkowicz et al. 2009 |
| *IL-6* | g.61T>C | PL | TT<br>BFT (sacrum I, C1, K1) ↓ | Szydlowski et al. unpubl. |
| *LEP* | g.3469 C>T | LW | BFT<br>Allele C ↓ | Jiang et al. 1999 |
| | | Landrace<br>Duroc<br>Yorkshire | no effect | Kennes et al. 2001 |
| | | PLW<br>PL<br>L990 | no effect | Szydlowski et al. 2004 |
| *LEPR* | g.232T>A | PL | TT<br>BFT (over shoulder) ↓ | Mackowski et al. 2005 |
| | STR<br>(CA)nCG(CA)n<br>(intron 3) | L990 | Allele 350 bp<br>BFT (over back, sacrum I, K1) ↓ | Chmurzynska et al. 2004 |
| *LIF* | c.2604A>G | LW × L | AA<br>BFT (at 10th rib) ↑ | Fan et al. 2009b |
| *MC4R* | c.707A>G<br>(Arg236His) | B × Y | Allele G (Yorkshire)<br>BFT (average, lumbar, at last rib) ↓ | Fan et al. 2009c |
| | c.892A>G<br>(Asp298Asn) | Duroc | Allele A<br>UBF, UIMF ↓ | Schwab et al. 2009 |
| | | PLW | Allele A<br>BFT (average) ↑ | Piorkowska et al. 2010 |
| | | LW × L | Allele A<br>BFT (at 10th rib) ↑ | Fan et al. 2009b |
| *MTHFR* | c.453A>G | LW × L | Allele G<br>BFT (at 10th rib) ↑ | |

**Table 5**. cont.

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| *PPARGC1A* | g.1105C>A (Arg369Arg) | PLW | CC<br>BFT (over back) ↓ | Stachowiak et al. 2007 |
| *PPARD* | 25 polymorphisms | GL | Haplotype 4<br>BFT (in the middle of the back) ↓ | Meidtner et al. 2009 |
| *RETN* | g.-178G>A | PLW | AA<br>BFT (average) ↓ | Cieslak et al. 2009 |
| *TNF*-α | g.6464C>T | PLW | TT<br>BFT (sacrum I & III, C1, K1) ↑ | Szydlowski et al. unpubl. |
| *TNNI1* | g.5174T>C | LW × M | Allele T<br>FP, BFT (thorax-waist, between 6th and 7th rib) ↑ | Xu et al. 2010 |
| *TNNI2* | g.1167C>T | LW × M | Allele C<br>FP, BFT (average, thorax-waist) ↓ | |
| *VDBP* | c.473-164C>T | LW × L | Allele T<br>BFT (at 10th rib) ↑ | Fan et al. 2009b |

B × Y = Berkshire × Yorkshire cross; BFT = backfat thickness; FP = body fat percentage; GL = German Landrace; IMF = intramuscular fat content; L = landrace; L990 = Polish Synthetic Line 990; LW × L = Large White × Landrace cross; LW × M = Large White × Meishan cross; LW = Large White; PL = Polish Landrace; PLW = Polish Large White; STR = short tandem repeat; UBF = ultrasonically measured 10th rib backfat; UIMF = ultrasonically measured intramuscular fat; IMF EBV = estimated breeding value for IMF

level variability. Studies performed with the use of porcine microarrays or the qPCR (quantitative PCR) approach on pigs differing in fatness traits have brought new information on a possible molecular mechanism responsible for this variability. For example, a hepatic transcription profile was analyzed using the porcine oligonucleotide microarray in 2 breeds – German Landrace and Pietrain – differing in growth rate, body composition, and nutrient utilization (Ponsuksili et al. 2007). In the leaner Pietrain pigs a significant up-regulation of genes involved in cell growth and/or maintenance, protein synthesis, and cell proliferation (*PPARD, POU1F1* and *IGF2R*) were found at the peripubertal age. On the other hand, in the fatter German Landrace pigs, key genes involved in lipid metabolic pathways (*FASN, ACSS2* and *ACACA*) were up-regulated. This study showed that phenotypic breed differences in terms of fat deposition in pigs do not result from the up-regulation of lipolysis pathways, as it happens in mice. A similar analysis of differentially expressed genes in backfat was performed to compare lean-type Landrace and fatty indigenous Chinese Taihu pigs (Li et al. 2008). The study revealed a differential expression of 25 genes, including genes playing an essential role in lipid metabolism (e.g. *FABP3, LPL, ME1, SCD*, and *UCP2)* in backfat of Landrace pigs during the period from 1 to 5 months of postnatal development. Those authors speculated that the metabolism in backfat is more active in Landrace pigs than in Taihu pigs, which is in accordance with lower ability for fat deposition in Landrace pigs in comparison with Taihu pigs.

In another investigation, conducted with the use of expression microarrays, a comparison was performed between pigs of the same origin (a cross of 2 lines), but differing in terms of IMF in the longissimus muscle (Liu et al. 2009). An assumption was made that the differences in gene expression levels observed in other studies might be a result of breed characteristics rather than variations in IMF content *per se*. Altogether, 29 genes were differentially expressed in pigs with low and high levels of IMF (1.3% vs. 4.6%, respectively) and among them 63% were up-regulated in the group with a high IMF. The differentially expressed genes encoded proteins involved in 8 functional categories: metabolic process (mainly protein biosynthesis), cell communication, response to stimulus, binding, chromatin assembly, transport, regulation of transcription, and cell proliferation. Surprisingly, the list did not include any of the well-known master regulators of adipogenesis and lipid accumulation, such as *PPARγ*, *C/EBPα*, and *SREBP1*. Contrary to studies performed by Ponsuksili et al. (2007), the obtained results suggested that in the longissimus muscle the protein turnover, rather than lipid metabolism, was involved in increased fatness. Indeed, the interpretation of results obtained from gene expression studies should be careful, as it was suggested by Stachowiak et al. (2010), who showed that the expression level of the adiponectin receptor 1 (*ADIPOR1*) gene in longissimus dorsi and semimembranosus is affected by a complex interaction of many factors, such as tissue, age, and breed.

Interesting results were shown in a study of 2 obese mouse lines, selected for high body weight, which were compared with lean control lines by using the murine expression microarray. It was revealed that altogether 77 genes were differentially expressed in epididymal fat tissue, but among them only several were involved in the reg-

ulation of energy homeostasis (Aksu et al. 2007). One of them was the leptin gene, which was also found to be differentially expressed in porcine fat tissue (Li et al. 2008). The microarray studies carried out in the pig and mouse suggest that genes involved in the obese phenotype development are species- and breed-specific.

Another approach used in functional genomic studies is the analysis of transcription levels of a priori selected candidate genes, with well-known functions across different breeds. Using real-time PCR, Lord et al. (2005) analyzed the mRNA level of adiponectin and its 2 receptors (*ADIPOR1* and *ADIPOR2*) in 2 fat depots of Chinese Upton Meishan, Ham Line, and Large White pigs. In visceral fat tissue there were no differences in the adiponectin transcript level between the breeds, while in subcutaneous fat tissue, the adiponectin mRNA level was significantly higher in the lean Ham Line in comparison to the fat Chinese Upton Meishan gilts. On the other hand, in subcutaneous fat tissue no breed differences were found for both *ADIPOR1* and *ADIPOR2* transcript levels, whereas in visceral fat, higher mRNA levels were found in Large White gilts when compared with Chinese Upton Meishan pigs. In another study, the mRNA level of the *PDK4* gene, involved in glucose oxidation in mitochondria of skeletal muscles, was analyzed using real-time PCR in longissimus dorsi of Western-type Yorkshire and indigenous Chinese Meishan (Lan et al. 2009). The *PDK4* gene expression was higher in Chinese Meishan than in Yorkshire pigs. It was assumed that the difference was due to the characteristics of Meishan muscles, which contain more slow-twitch fibres. These fibres are believed to be more tender and savoury, and are rich in mitochondria and mitochondria-related proteins.

However, it must be emphasized that the results coming from transcriptome analysis require further verification, using proteomic studies and other 'omics' approaches to reveal key factors involved in adipogenesis (Prokesch et al. 2009).

## Searching for new sources of genetic variance for fatness traits

Extensive studies focused on functional or associated gene polymorphisms, in relation to human and mouse polygenic obesity and livestock fatness, are not very successful until now due to the very complex genetic background of these traits. Thus, other mechanisms have been considered to identify other sources of the genetic variance. Among them, the role of copy number variation and RNA interference seem to be especially interesting. In addition, also the so-called nuclear architecture may play an important role, since it was revealed that spatial position of genes involved in adipogenesis in nuclei of differentiating in vitro adipocytes is associated with their level of transcription (Szczerbal et al. 2009).

### Copy number variation

Copy number variants (CNVs) are defined as variable numbers (comparing with reference genome) of repeated DNA segments, whose length is from 1 kilobase to several megabases (Feuk et al. 2006). Sha et al. (2009) reported recently that CNVs can be genetic markers associated with BMI. Those authors showed such an association for CNVs in human chromosome 10q11.22. A low number of variants (1 copy or none) was connected with higher BMI. It might be caused by a number of gene copies located within this CNV region. One of them is *PPYR1*, which encodes a well-known neuropeptide Y playing a crucial role in regulation of food intake.

Also in the mouse a significant impact of the CNV polymorphism on gene expression was described. Analysis of 19 murine CNVs revealed that the expression level of over 80% of genes, covered by the CNVs, was correlated with the observed copy number (Orozco et al. 2009). Moreover, it was found that some of the CNVs, spanning certain genes (e.g. *Itlna, Csf2ra* and *Defcr-rs1*), were associated with abdominal fat and body weight. Phenotypic effects of the porcine CNVs have not been studied yet, but a preliminary information on their distribution is already available (Fadista et al. 2008).

### RNA interference

The importance of miRNAs in lipid metabolism has been documented several times. Among 80 murine miRNAs expressed in preadipocytes, 21 were expressed at various levels, depending on cell maturity (Kajimoto et al. 2006). Moreover, expression of some human miRNA genes (hsa-miR-325, hsa-miR-181a, and both hsa-miR-325, and hsa-miR-99a) is significantly correlated with the level of circulating leptin (LEP), adiponectin (ADIPOQ), and interleukin 6 (IL6), respectively (Klöting et al. 2009). Unfortunately, that study did not reveal any link between these miRNAs and putative target sequences. Actually it is known that a target sequence for hsa-miR-181a occurs in 3'UTR of the *ADIPOQ* gene, as it was revealed with the use of TargetScan software (http://www.targetscan.org). Also lipid

metabolism in *Drosophila* is regulated by miRNA. The depletion of miR-14 causes an unusually high level of di- and triacylglycerols (Xu et al. 2003). Special attention should be paid to the structure and expression of genes encoding miRNA and polymorphism of the target sequences in 3'UTR of the controlled genes. Since numerous miRNAs are conserved across vertebrates, a reliable identification of the conserved miRNA homologues in the pig genome is possible. It is estimated that approximately 10% of miRNAs are taxon-specific and their annotation requires complete genome information (Reddy et al. 2009). Until now, more than 70 porcine miRNAs have been annotated in the miRNA database (miRBase), but much more were identified in the pig genome by expression studies (Kim et al. 2008; Huang et al. 2008; Reddy et al. 2009; McDaneld et al. 2009).

The number of putative target sequences in 3'UTR can be quite high. For instance, among tools for the detection of miRNA target sequences, MicroInspector software displays 29 sequences in the *SCD* gene, corresponding to 22 pig miRNAs annotated in the miRBase. Unfortunately, different softwares sometimes produce results that are not coherent, so selection of sequences of interest can be difficult (Landi et al. 2008). TargetScan software seems to be a more valuable tool, because it can detect miRNA binding sites that are conserved broadly among vertebrates or among mammals, e.g. 9 out of 11 target sequences present in the human *SCD* gene occur in the porcine *SCD* gene.

## Conclusions

In the pig genome a large number of QTLs for fatness traits have been identified, which span hundreds or more of candidate genes (Schook et al. 2005). Thus it is not surprising that studies of associations of selected candidate gene polymorphisms are progressing slowly. Moreover, to date the comparative genomics approach in selecting candidate genes for pig fatness has appeared not to be very efficient. The large number of genes involved in the determination of polygenic fatness/obesity traits and their relatively small effects, as well as interactions between genes (i.e. epistasis) are probably responsible for this situation. The meta-analyses of human predisposition to obesity seem to support this hypothesis, since the effect of strong candidates (*FTO, MC4R* or *INSIG2*) is small or even doubtful (Lyon et al. 2007; Willer et al. 2009). Also studies of polymorphisms of 2 porcine candidate genes (*MC4R* and *FTO*) revealed no strong association with fatness. It must be emphasized that studies on human obesity are focused on BMI, while searching for markers of pig fatness is concerned with different fatness traits, directly measured after slaughter. This may cause limitations for the comparative genomics approach, since candidate genes for human obesity are not necessarily relevant candidates for IMF, BFT or FAC, for example. Taking the above into consideration it can be postulated that in the future, comprehensive studies with the use of structural and functional genomics (including epigenetic modification) may bring new insight into pig fatness genetics.

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