CHAPTER EIGHT

Genetics of Adiposity in Large Animal Models for Human Obesity—Studies on Pigs and Dogs

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Contents

Abstract

The role of domestic mammals in the development of human biomedical sciences has been widely documented. Among these model species the pig and dog are of special importance. Both are useful for studies on the etiology of human obesity. Genome sequences of both species are known and advanced genetic tools [eg, microarray SNP for genome wide association studies (GWAS), next generation sequencing (NGS), etc.] are commonly used in such studies. In the domestic pig the accumulation of

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adipose tissue is an important trait, which influences meat quality and fattening efficiency. Numerous quantitative trait loci (QTLs) for pig fatness traits were identified, while gene polymorphisms associated with these traits were also described. The situation is different in dog population. Generally, excessive accumulation of adipose tissue is considered, similar to humans, as a complex disease. However, research on the genetic background of canine obesity is still in its infancy. Between-breed differences in terms of adipose tissue accumulation are well known in both animal species. In this review we show recent advances of studies on adipose tissue accumulation in pigs and dogs, and their potential importance for studies on human obesity.

1. INTRODUCTION

Long-lasting breeding of domestic animals has resulted in the creation of a wide variety of breeds, differing in terms of numerous traits, related to morphology, behavior, coating, productivity, quality of animal products, adaptation to abiotic (eg, temperature) and biotic (eg, resistance to specific pathogens) environmental conditions, etc. The observed phenotypic variation between breeds of the same species reflects differences in their gene pools.

Breeding strategies, mainly based on selection, are quite different in livestock and companion species. In livestock the most important are production (meat, milk, wool, eggs, etc.) and the related functional traits (fertility, longevity, adaptation to specific environmental conditions, etc.). Thus, a directional selection is focused on continuous improvement of economically efficient production of high quality animal products by healthy animals managed under welfare conditions. A crucial step of selection in mammalian livestock (eg, cattle and pigs) is evaluation of breeding value of males, carried on the basis of their offspring performance (eg, milk yield, daily body mass gain, fatness traits, etc.). In this procedure, called progeny testing, sophisticated statistical methods are used. Recently a new approach, based on the use of SNP microarrays and called genome selection, has been implemented into this methodology. In contrast, a stabilizing selection in accordance with breed standards is applied in companion species, mainly dogs and cats. In this approach, called pedigree selection, a crucial role is played by pedigree information, concerning phenotypes of the ancestors, including prizes won on animal shows.

The global number of livestock and companion animal breeds is large and the exact figures remain unknown. It is estimated that several hundreds of pig breeds have been established worldwide. At the Pig website (http://www.thepigsite.com) 75 common breeds are specified, but this database does not

include a wide variety of local breeds. For example, in the United Kindom there are 18 autochthonous breeds (the British Pig Association, http://www.britishpigs.org.uk/), while in China 48 native breeds are recognized (http://www.Pigprogress.net). In the case of dogs this knowledge is more precise due to an international database run by Fédération Cynologique Internationale (FCI) (http://www.fci.be/en/). According to this database, approx. 340 dog breeds are internationally recognized. It is also important to point out that among domestic mammals an exceptional phenotypic variability is observed in dogs.[1]

Due to the extensive use of selected sires in artificial insemination (eg, in cattle and pigs) or a limited number of founders of a breed (numerous dog breeds) a random increase of undesired mutations in a gene pool may occur. Among domestic mammals the highest number of monogenic diseases with a known causative mutation was described in the dog.[2] Importantly, a majority of these diseases have counterparts in humans, and thus the dog has become an important large animal model for human biomedical science.[3] Knowledge on complex diseases (eg, diabetes, metabolic syndrome, obesity, cardiovascular diseases, neoplasms, etc.) in livestock and companion species is different, due to the difference of their life span. In addition, attentive veterinary care, common in the case of dogs and cats, facilitates a precise diagnosis of such diseases.

Among domestic mammals two species, namely the pig and the dog, are crucial models for human obesity. The pig is an omnivorous species showing a wide range of anatomical, histological, and physiological similarities to humans,[4] while the dog is a carnivorous species sharing with the human the same environment, including diet and life style, for example, physical activity.[5] Deposition of adipose tissue in livestock species is an important production trait, influencing meat quality and its dietetic value, as well as fattening effectiveness.[6] Thus, mechanisms responsible for nonpathogenic accumulation of subcutaneous, visceral, or intramuscular fat tissue in these species, including pigs, have been extensively studied. The pig is a model of special interest because similarly to humans, an excessive accumulation of adipose tissue is responsible for the development of obesity-related diseases. This knowledge may help us to understand a known relationship between specific fat depots (eg, abdominal) and human obesity-related diseases, that is, diabetes.[7] Recently, it was shown that diet-induced obesity in pigs can be a model in studies on the effect of obesity on the development of metabolic syndrome.[8] Also physiological indices related with obesity are similar in both species, that is, a high serum concentration of low density lipoproteins (LDL) and a low high density lipoprotein (HDL) concentration.[9]

Studies on adiposity of companion animals (dogs and cats) have recently received special attention because obesity is considered as an emerging health problem in these species.[10] The major factors affecting canine and human obesity are similar: nutrition, physical activity, and hereditary predisposition.[11] An analysis of over 2000 dogs living in Beijing (China), aimed on the identification of risk factors for obesity indicated the following ones: feeding (the use of noncommercial food type and increased feeding frequency), age, physical activity, neutering, breed, and sex.[12] Another similarity between human and canine obesity was also endocrinologically documented in terms of the relationship between adiposity and the level of adipokines.[13–15]

The hereditary background of adipose tissue accumulation may be analyzed in terms of the effect of breed, revealing chromosomal regions [quantitative trait locus (QTL)] harboring the predisposing DNA variants, identification of the associated gene polymorphisms and deciphering variation of gene expression, including epigenetic mechanisms. Such studies have been extensively carried out with regard to porcine fatness traits (reviewed by Switonski et al.[16]), while in dogs they are in infancy.[17,18] This review presents recent advances, focused on the importance of these studies to provide insight to the genetic background of human obesity.

2. BREED SPECIFIC DIFFERENCES OF ADIPOSE TISSUE ACCUMULATION

Variability of fat tissue deposition between pig breeds, as well as predisposition to obesity in some dog breeds are well-known phenomena. Similarly to the human, heritability of pig adiposity fluctuates around 0.5.[16] It may be assumed that also in dogs this coefficient has a similar value.

Commercial pig breeds are classified as a meat type, since accumulation of fat tissue is accepted on a relatively low level, essential for high quality of meat. However, there are numerous autochthonous breeds, representing a fat-type pig, which accumulate an excessive amount of adipose tissue. These include well-known European breeds—Mangalica[19] and the Iberian pig,[20] as well as several Chinese breeds, including the Erhualian breed.[21] One can speculate that predisposition to adipose tissue accumulation in these breeds, also referred to as the thrifty genotype, is an adaptive mechanism to uneven availability of feed. A classic example of this genotype is a miniature Ossabaw breed, which develops morbid obesity and metabolic syndrome under a calorie-rich diet.[22] This breed is considered to be a very useful large animal

model in studies on human obesity and obesity-related diseases, for example, metabolic syndrome.[23–25]

There are several adipose traits, which have been extensively studied in pig breeds due to their importance for quality of meat and efficiency of fattening. These traits are usually studied *postmortem*, when carcass dissection is carried out. Among them the most crucial include thickness of subcutaneous fat tissue on the back at different locations [back fat thickness (BFT), expressed in cm abdominal fat weight (AFW, expressed in kg), intramuscular fat content (IMF, expressed in percentage)] and fatty acid composition of fat tissues. These analyses provided detailed insights into mechanisms related with adipose tissue accumulation. It is well known that some breeds have a very thin subcutaneous fat tissue (eg, the Pietrain breed), in a majority of breeds this tissue is moderately thick (eg, breeds belonging to Large White breeds), while in some breeds this depot is very thick (eg, the Mangalica breed). For example, a comparison of BFT of Mangalica and Hungarian Large White, slaughtered at the same body weight (approx. 130 kg), showed a significant difference of the average thickness, of 5.0 and 2.5 cm, respectively.[26] Another example concerns the influence of breed and production system on performance traits, including BFT and mass of perineal fat. Pigs of two breeds, the commercial Large White (meat-type breed) and Basque (autochthonous, fat-type breed), were fed the same growing diet and slaughtered at the same body weight (on average 145 kg). It was found that the accumulation of adipose tissue was significantly higher in Basque pigs. The average BFT and perineal fat mass in Basque pigs were 4.7 cm and 4.8 kg (in the conventional indoor housing system), or 5.0 cm and 4.8 kg (indoor housing system with free access to outdoor area), respectively. These values for Large White pigs were 2.3 cm and 1.9 kg, or 2.4 cm and 1.9 kg, respectively.[27] Differences of porcine fatness is also manifested on the cellular level. It has been found that the size of adipocytes is larger in fat breeds than in leaner ones. For example, the commercial Landrace breed with a low backfat thickness has smaller adipocytes in the subcutaneous adipose tissues when compared with the fat-type Meishan breed.[28] Moreover, tissue-specific differences in adipocyte size are also observed. Adipocytes derived from intramuscular fat tissue are smaller than those from subcutaneous and visceral fat tissues. The aforementioned examples clearly show that gene pools involved in adipose tissue accumulation are different in the compared breeds.

Obesity in dogs is an emerging health problem, which has received serious attention of veterinarians, breeders, and owners. Evaluation of adipose tissue accumulation in dogs is usually performed with the use of a

Table 1 Prevalence (%) of Obesity and Overweight in Dogs in the USA (http://www.petobesityprevention.org/pet-obesity-fact-risks/).

Year	Obese Dogs, BCS = 5	Overweight Dogs, BCS = 4	Total
2011	21.5	31.5	53.0
2012	15.7	36.8	52.5
2013	16.7	35.9	52.6
2014	17.6	35.1	52.7

subjective 5- or 9-level body condition score (BCS) scale. In the 5-level scale the following BCS values mean: 3, normal body condition, 4, overweight, and 5, obesity. It should be pointed out that more objective methods are also available, for example, dual-energy X-ray absorptiometry (DEXA) and deuterium oxide (D_2O) dilution.[29] Interestingly, the results of the DEXA approach very well correlate with the subjective BCS scale.[30] The incidence of overweight and obesity is constantly increasing. It was estimated by the Association for Pet Obesity Prevention that in the USA the prevalence of obesity and overweight in dogs exceeded 52% (Table 1). It is also well known that some breeds are predisposed to obesity. A wide survey of 21,754 dogs in the USA showed the highest prevalence of obesity in Beagles (47.7%), Cocker Spaniels (46.3%), Shetland Sheep Dog (45%), Golden Retrievers (44.3%), and Labrador Retrievers (41.1%).[11] Another study, comprising 2391 dogs from Beijing (China), showed a very high overall prevalence of adiposity (44.4%), with the highest rates noted in Pugs (70.7%), Cocker Spaniels (69.4%), Pomeranians (54.6%), Pekingese dogs (51.9%), Golden Retrievers (51.9%), Chihuahuas (46.9%), and Labrador Retrievers (46%), while the lowest rate was observed in Huskies (25%), Miniature Poodles (23.9%), and Poodles (20.3%).[12]

These observations indicate that the presence of genetic variants predisposing to an excessive accumulation of adipose tissue in gene pools of the aforementioned breeds are different. Thus, breeds differing significantly in terms of their predisposition to obesity are of special interest when searching for the predisposing and protecting genetic variants.

3. EPIGENETIC MARKERS OF ADIPOGENESIS AND ADIPOSE TISSUE

Development of adipose tissue is a consequence of two processes, namely generation of new adipocytes—hyperplasia, and an increase in their

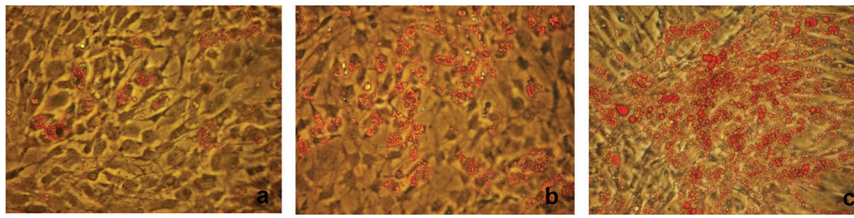

Figure 1 Accumulation of lipid droplets during porcine in vitro adipogenesis. Oil Red O staining was performed at days 3 (A), 7 (B), and 14 (C) of the differentiation. *Photo: I. Szczerbal.*

volume—hypertrophy.[31] Thus, genes involved in adipocyte differentiation or lipid metabolism are considered to be functional candidates for porcine fatness traits.[32,33] A majority of model studies on adipogenesis were carried out on mice, while knowledge about this process in large animal models is limited in the pig and very scarce in the dog. Since the pig is considered as a valuable model organism for studies on human obesity, understanding genetic and epigenetic mechanisms governing porcine adipocyte differentiation is highly required.

Adipogenesis in the pig (Fig. 1), similar to other species, is regulated by a complex network of transcription factors, among which PPARγ and members of the C/EBP family play a major role.[34] A number of other positive and negative regulators of porcine adipogenesis have been described.[35,36] Interestingly, there are transcription factors (eg, KLF13), which showed no effect on mouse adipogenesis, but have been recognized as proadipogenic factors in the pig.[37] In recent years increasing attention has been addressed to epigenetic factors involved in porcine adipogenesis, including DNA methylation, the role of noncoding RNA (ncRNA) and nuclear architecture. It is assumed that epigenetics is an important contributor of "the missing heritability" in complex traits.[38]

3.1 DNA Methylation

DNA methylation has gained special interest among epigenetic mechanisms involved in the development of human obesity. Different approaches concerning global, gene-specific, and genome-wide DNA methylation levels were applied to search for its role in obesity. New methodologies such as epigenome-wide association studies (EWAS) allowed researchers to expand these studies in recent years. Changes in DNA methylation were observed in a number of candidate genes in the human, which functionally are linked

mainly with adiposity or appetite control, but also other cellular processes. In addition, obesity associated differentially methylated (DM) sites have been identified with the use of genome-wide methods.[39] There are examples showing that perinatal nutritional exposures can cause epigenetic consequences in the offspring.[40] Moreover, the DNA methylation profile is not stable throughout adult life, but may be changed by different interventions, such as exercise,[41] diet,[42] and weight loss surgery.[43]

A comprehensive study of three pig breeds differing in terms of their fat accumulation, that is, the Landrace (a lean, meat type), Tibetan (a feral type) and Rongchang (a fat type), produced a DNA methylation map for adipose and muscle tissues.[44] Altogether, 10 samples from various anatomic locations were analyzed: 8 adipose tissues (subcutaneous, 3; visceral, 4; intermuscular, 1) and two skeletal muscle tissues (the longissimus dorsi muscle and psoas major muscle). Differentially methylated regions (DMRs) were identified and their number varied between breed, tissues, and sexes. Analyses of breed specific DMRs in promoters of adipose tissues revealed that the Rongchang and Tibetan breeds are phylogenetically closer to each other than the Landrace, while the Landrace is closer to Tibetan than the Rongchang breeds when muscle tissues were evaluated. This indicates that breed-specific differences are found not only on the genetic, but also the epigenetic level. DMRs in promoters were also tissue-specific and methylation in these genomic elements correlated with adipose tissue from different locations. For example, intermuscular adipose tissue was more similar to visceral adipose tissue in terms of the methylation pattern. Among 282 porcine gene orthologs to human obesity-related genes, 80% has shown location within the identified DMRs. Also more than 70% of porcine genes from the QTL regions affecting fatness and pork quality overlapped with the identified DMRs.

A detailed analysis of methylated genes from different adipose depots (the same samples as in Li et al.[44]) has shown that comethylated genes from visceral and intermuscular adipose tissues are associated with inflammatory and immune processes, while comethylated genes in subcutaneous adipose tissue are mainly associated with metabolic processes.[45] These observations were also confirmed by a comprehensive genome-wide comparison of gene expression profiling.[46] In addition, different layers of porcine backfat tissue (superficial vs. deep) were studied in terms of DNA methylation and DMRs associated with differentially expressed genes involved in lipid metabolism and regulation of immune-related cytokines have been reported.[47] Distinct features of adipose tissues from various locations identified on molecular

levels reflect their functional and metabolic differences. With the use of transcriptomic studies it has been recognized that both visceral and intramuscular adipose tissues are associated mainly with immune and inflammation responses and these tissues have been identified as a metabolic risk factor for obesity.[48,49]

3.2 ncRNA

Two categories of noncoding RNAs, that is, microRNA (miRNA) and long noncoding RNA (lncRNA), have been recognized as important regulators of gene expression. The role of miRNA in mammalian adipogenesis has been recently reviewed by Peng et al.[50] A number of proadipogenic and antiadipogenic miRNAs have been identified. A comprehensive study concerning porcine miRNAs involved in adipogenesis and a comparison of miRNAomes during differentiation of intramuscular (IVSC) and subcutaneous vascular stem cells (SVSC) were performed by Guo et al.[51] The authors identified 224 known and 280 potential porcine miRNAs and showed expression similarities and differences during differentiation of IVSC and SVSC. The miRNome of back fat in two adult Italian Large White pigs was also analyzed by Gaffo et al.[52] Unfortunately, in the aforementioned studies no specific miRNAs associated with the accumulation of adipose tissue were indicated.

In recent years it has been shown that also long-noncoding RNAs (lncRNA) may play an important role in murine adipogenesis.[53] So far, lncRNAs have not been extensively studied in the pig. There is only a single study concerning antisense long noncoding RNAs (AS lncRNAs), which revealed that a novel AS lncRNA (PU.1) has a positive effect on porcine adipogenesis.[54] The authors hypothesized that modulation of lncRNA may provide a new target for the control of fat accumulation.

3.3 Nucleus Architecture

Regulation of transcription is also controlled by nuclear architecture. It was shown that the arrangement of key adipogenesis genes and chromosomes harboring these genes within a nuclear space have specific patterns during different stages of in vitro adipogenesis in pigs (Fig. 2). The radial nuclear position of selected genes (eg, *PPARG*, *GATA2*, *SREBF1*) has changed during differentiation and these changes correlated with the transcriptional status, as upregulated genes were more internally located within nuclei.[55] Further studies revealed spatial coassociation of genes activated during

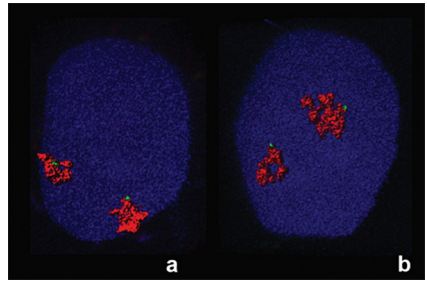

Figure 2 Changes of nuclear architecture during porcine in vitro adipogenesis. Distribution of the *FABP4* gene and SSC4 chromosome territories in a mesenchymal stem cell (A) and an adipocyte from day 14 of differentiation (B) are shown. *Photo: I. Szczerbal.*

porcine adipogenesis with nuclear speckles (SC-35). It was suggested that it can be a mechanism which enhances gene expression.[56] On the other hand, analysis of the nuclear architecture of matured adipocytes derived from fat tissue has shown that correlation between nuclear position and transcription activity is not common in case of genes involved in lipid metabolism.[57] It has been suggested that the relationship between transcriptional status of adipogenic genes and their nuclear positioning is characteristic for differentiation process, when genes switch from the silent to the active state.

3.4 Nutrigenomics and Epigenetic Modifications

There is a growing body of evidence that epigenetic modifications may be caused by environmental factors, including nutrition.[58] Many studies have shown that dietary interventions during gestation induce changes in DNA methylation and histone modification patterns of offspring. Different types of diets (eg, high-fat, high-protein, or undernutrition) as well as supplementation or restriction with a range of dietary factors, including those which affect the methionine cycle (eg, folate, vitamin B6 and B12), were examined. Moreover, nutrition during pregnancy has not only the direct effect on a fetus, but may also show a transgenerational effect.[59] Rodent models, but also farm animals have been widely used in such experiments.[60]

An example of a transgenerational epigenetic effect in pigs was reported by Braunschweig et al.[61] Three generations of Large White pigs were studied in terms of heritable epigenetic changes caused by a methyl-enriched diet. Males from the F0 generation were fed with a diet supplemented with high amounts of methylating micronutrients and the control group received a standard diet. The feeding effect was analyzed in the F2 generation. It was

found that F2 animals had a lower fat percentage and a higher shoulder muscle percentage as compared to the controls. Moreover, differences in transcript levels of genes from the liver and muscle tissue as well as DNA methylation changes in the *IYD* (iodotyrosine deiodinase) gene were found in that experiment.

The effects of maternal diet on transcriptional regulation of the *MSTN* (myostatin) gene, a negative regulator of skeletal muscle growth, was studied in Meishan pigs.[62] This indigenous breed is traditionally raised on a low-protein diet. Sows were fed with low-protein or standard-protein diets throughout gestation and lactation periods. The *MSTN* transcript level was analyzed in the longissimus dorsi muscle of their offspring at weaning (approx. body weight 20 kg) and finishing stages (approx. body weight 40–70 kg). The *MSTN* expression was affected in pigs exposed to a maternal low-protein diet at the finishing stage through regulatory mechanisms, including histone modification and microRNA activity. It has been shown that the immediate effect is not related with epigenetic regulations, while the long-term effects were epigenetically controlled. The impact of the maternal low-protein diet during gestation and lactation was also investigated in Meishan pigs in relation to hepatic cholesterol metabolism.[63] Piglets at weaning had a lower body weight and liver weight. The phenotypic changes were associated with upregulation of hepatic genes through epigenetic modifications.

Nutrigenomics studies were also carried out in dogs, but they were focused on the impact of different diets and supplementations on transcript levels of key genes related with obesity.[18] However, to date no reports on the influence of dog nutrition on epigenetic modifications have been presented.

4. QTL FOR ADIPOSITY TRAITS

Discovery of common DNA markers, mainly microsatellites [short tandem repeats (STR), short simple repeats (SS)] has facilitated development of high density marker genome maps. In the early 1990s international marker genome map projects were launched and they were focused on mapping microsatellite markers. The most important porcine projects were established in Europe [the European Pig Gene Mapping Project (PiGMaP)] and the USA [the porcine linkage map supported by the United States Department of Agriculture (USDA)]. In 1993 an international consortium DogMap was launched for the development of the dog genome marker map. Within a relatively short time advanced maps, comprising thousands of mapped STR

markers, were presented. The porcine maps were extensively used in the so-called genome scanning, aimed at the identification of chromosomal regions [quantitative trait loci (QTLs)] harboring markers linked with unknown DNA variants influencing variability of numerous production traits, including fatness. For this purpose several reference families were established, in which founders differed phenotypically. Some of these families were created by crossing commercial meat-type breeds (eg, Large White, Duroc, Landrace) and local fat-type breeds, for example, Mangalica.[21,64,65]

The next step in deciphering genome organization was its sequencing. The genome sequence of the dog was described in 2005 by Lindblad-Toh et al.,[66] while the porcine one in 2012 by Groenen et al.[67] The genome sequencing projects revealed the presence of numerous single nucleotide polymorphisms (SNPs) in the studied genomes and it facilitated development of SNP microarrays, comprising up to 170k SNPs for the dog and 60k SNPs for the pig. It is expected that soon a high density porcine microarray (510k) will be commercialized. These powerful tools were extensively used in the so-called genome-wide association study (GWAS) for QTL studies in pigs and the identification of gene mutations responsible for canine monogenic diseases, as well as phenotypic variation between breeds.

Genome scanning resulted in the detection of numerous QTLs for adipose tissue accumulation in the pig. In the PigQTL database (http://www.animalgenome.org/cgi-bin/QTLdb/SS/index), among the reported 13,030 QTLs for 663 pig traits there are 243 regions for an average backfat thickness, 180 for IMF and 34 for AFW. This summary clearly shows that the genetic background of adipose tissue accumulation in pigs have been extensively studied.

The first QTL for pig fatness traits was published over 20 years ago.[68] This region, later called *FAT1*, was assigned to pig chromosome 4 [Sus scrofa chromosome 4 (SSC4)], but the search for a candidate gene in this region is still not completed. Recently, a novel candidate gene—*PLAG1* (pleiomorphic adenoma gene 1) has been proposed.[69] Interestingly, this gene has been under a strong selection during pig domestication, since distinct sequence differences, related with body size, were found between the domestic pig and the wild boar.[70]

According to the PigQTL database, QTL regions for fatness traits are distributed on all chromosomes, but their significance is not similar. During the last 5 years a detailed study on QTLs for porcine fatness traits confirmed the importance of regions residing on the following chromosomes: SSC2,[21,69] SSC4,[21,69] SSC7,[21,69,71] and SSCX.[21,69,72]

An interesting comparative whole genome search for candidate genes involved in lipid metabolism, performed with the use of the next generation sequencing (NGS) approach, was presented by Molnar et al.[73] The authors compared the genomes of three males of Mangalica, representing three color variants (blond, red, and swallow-belly), with the genome of the commercial meat-type Duroc pig and the reference pig genome. The comparison revealed nonsynonymous SNPs that were found in Mangalicas, but were not present in the Duroc pig in 52 genes involved in lipid-related metabolic processes and 49 of them were localized within fat-related QTLs. Interestingly, among the 52 highlighted genes, 42 were not previously associated with fatness traits. A comparison of this gene list with the set of 97 human genes associated with predisposition to obesity, presented by Locke et al.,[74] revealed that none of the 52 pig candidate genes was indicated in the human.

A vast majority of QTL studies were carried out with the aim to identify chromosomal regions for production traits; however, there are also reports on comparative genome analyses of human and porcine genomes focused on the hereditary background of adiposity. A whole genome comparison of obesity related traits in the human [subscapular skinfold thickness (SUB)] and the pig (BFT), based on genotypes obtained with the use of SNP microarrays—porcine 60k and human 500k, was reported by Kim et al.[75] This analysis showed that human chromosome 2 (HSA2), which is orthologous to pig chromosomes SSC3 and SSC15, harbors loci (*MRPL33*, *STK39*, *ZNF385B*, *PARD3B*, and *ERDB4*), which variants probably predispose to obesity. However, until now no reports confirming the role of these genes have been published. Moreover, these genes were not indicated as predisposing to human obesity.[74]

The GWAS methodology has been widely applied in studies on behavioral, morphological traits as well as complex diseases, including cancers.[76] Several studies concerned the identification of QTLs for body size. Using the GWAS approach the phenotypic variation between dog breeds was studied by Vaysse et al.[77] The authors identified 44 genomic regions responsible for extreme phenotypic differentiation across 46 breeds. One of the analyzed traits was body weight of normal (standard) dogs. In the identified region on chromosome CFA15 the *IGF1* gene is present. This finding confirmed an earlier study of Sutter et al.,[78] who showed that different gene variants of this gene are characteristic of large and small breeds. Unfortunately, until now there have been no reports on the use of GWAS to study canine obesity.

5. POLYMORPHISMS ASSOCIATED WITH FAT TISSUE ACCUMULATION

Extensive studies with the use of GWAS technology, as well as sequencing of functional and positional candidate genes resulted in the identification of polymorphisms associated with pig fatness, while in dogs such analyses are not so advanced. The associated SNPs are predominant; however, in recent years also some copy number variations (CNV) appeared to be associated with pig fatness and dog obesity.

5.1 SNPs and Indels in Pigs

Identification of genetic variants associated with lipid metabolism and fat tissue accumulation is an important goal of pig breeding, presently focused on fast growing and lean, meat-type pigs. The most favorable fatness-related traits in pig breeding include low back fat thickness (BFT, cm) and abdominal fat weight (AFW, kg), while a moderate intramuscular fat content (IMF, %) or marbling score (subjectively evaluated deposition of intramuscular fat) improve sensory parameters of meat. Studies of candidate genes, known as predisposing humans to obesity, often did not confirm similar evidence in pigs or other domestic mammals.

The candidate gene approach, based on the function of the gene, preferably supported by data from a GWAS or QTL mapping, is the most common strategy in searching for genes with a potential phenotypic effect. An extensive study of human obesity in multiple populations showed 97 loci, significantly associated with predisposition to obesity.[74] Among genes located nearest five most significant markers there are loci of *FTO* (fat mass and obesity associated), *MC4R* (melanocortin 4 receptor), *TMEM18* (transmembrane protein 18), *GNPDA2* (glucosamine-6-phosphate deaminase 2), *GABRG1* (gamma-aminobutyric acid A receptor, gamma 1) and *SEC16B* (SEC16 homolog B, endoplasmic reticulum export factor) genes. Two of these genes, namely *FTO* and *MC4R*, have also been studied in pigs (Table 2).

The *FTO* gene encodes a nuclear protein alpha-ketoglutarate-dependent dioxygenase, which physiological function remains unknown. The most extensively studied variant of this gene, SNP in intron 1, has been repeatedly indicated as associated with an increased risk of obesity (elevated BMI) in multiple human populations. The molecular mechanism of this phenomenon, an interaction between the enhancer region harboring the SNP and

Table 2 Recent Reports (Not Included in the Review by Switonski et al.[16]) on the Association of Polymorphism in Selected Pig Candidate Genes With Fat Tissue Accumulation.

Gene	Polymorphism (Localization)	Breed	Traits Showing an Association	References
Genes, which human orthologs are associated with predisposition to obesity[74]				
FTO	g.276T > G	Italian Duroc	BFT	[79]
		Commercial pigs	BFT IMF	[79]
	c.594C > G FM244720:g.400C > G (exon3)	Meishan × Pietrain	AFW BFT	[80]
		Commercial crossbred pigs	BFT Fat in the belly IMF	[80]
	g.−167T > G (5′ flanking region)	Polish Landrace	BFT AFW	[81]
		Polish Large White	ns	[81]
		Synthetic line 990	ns	[81]
	A227G	Suzhong	Marbling score	[82]
MC4R	c.1426A > G, p.Asp298Asn (exon 1)	Duroc × Iberian	ns	[83]
		Italian Large White	BFT	[84]
		Italian Duroc	ns	[84]
		Synthetic line DIV_2	BFT	[85]
		Commercial pigs	IMF	[86]
		Duroc	BFT	[87]

(*Continued*)

Table 2 Recent Reports (Not Included in the Review by Switonski et al.[16]) on the Association of Polymorphism in Selected Pig Candidate Genes With Fat Tissue Accumulation.—cont'd.

Gene	Polymorphism (Localization)	Breed	Traits Showing an Association	References
Genes encoding adipokines and their receptors				
ADIPOQ	c.−67G > A	Polish Landrace	ns	[88]
	c.−106_−91delGCCAGGGGTGTGAGCC (promoter)	Line 990		
LEP	c.3469T > C (exon 3)	Synthetic line DIV_2	ns	[85]
		Duroc	ns	[87]
	g.1387C > T (intron 2)	Iberian × Landrace cross	BFT	[89]
	g.+846C > T (3′UTR)	Polish Landrace	AFW	[90]
LEPR	c.2002C > T	Duroc × Iberian cross	BFT	[83]
	(described previously as c.1987C > T; p.Leu663Phe)		IMF	
		Iberian × Landrace cross	BFT	[89]
	(exon 14)		IMF	
		Duroc	BFT	[91]
		Duroc	BFT	[87]
RETN	AM:157180:g.1473A > G (p.Ala36Thr)	Landrace × Chinese-European	IMF	[92]
Other candidate genes				
ACACA	c.*99A > T (3′UTR)	Polish Large White	AFW	[93]
			BFT	
		Polish Landrace	BFT	[93]
	c.*195C > A	Polish Landrace	BFT	[93]
ACSL4	G2645A (3′UTR)	(Landrace × Yorkshire) × Duroc	IMF	[94]

BMP5	c.*131C > T (3′UTR)	Large White × Meishan	BFT	[95]
CART	T415 C (intron 1)	Landrace × Lantang F_2 population	BFT IMF	[96]
	C640 T (intron 2)	Landrace × Lantang F_2 population	BFT	[96]
	T847 C (intron 2)	Landrace × Lantang F_2 population	BFT	[96]
GNAS	g.324C > T (exon 10) and rs196952953 haplotype	Landrace	IMF	[97]
HNF1A	c.327 – 13A > G (intron 1)	Berkshire × Yorkshire	BFT	[98]
	c.1768 + 40_23del (intron 9)	Berkshire × Yorkshire	BFT	[98]
ME1	c.*488A > G (3′UTR)	Polish Large White	BFT	[99]
		Polish Landrace	BFT	[99]
MSTN	g.435G > A and g.447A > G haplotype (promoter)	Duroc and Landrace	BFT	[100]
NAMPT	AM999341:g.669T > C (intron 9)	Wild boar × Meishan	BFT	[101]
		Landrace × Chinese-European synthetic population	ns	[101]
SCD	c.*2041T > C (3′UTR)	Berkshire	IMF	[102]
	c.−353C > T (5′flanking)	Polish Large White,	ns	[103]
	c.−233T > C (5′flanking)	Line 990		
	c.*164A > G (3′UTR)			
	c.*928G > C (3′UTR)			
	c.*2574_257delGTC (3′UTR)			

(*Continued*)

Table 2 Recent Reports (Not Included in the Review by Switonski et al.[16]) on the Association of Polymorphism in Selected Pig Candidate Genes With Fat Tissue Accumulation.—cont'd.

Gene	Polymorphism (Localization)	Breed	Traits Showing an Association	References
PCSK1	g.1696C > A (intron 4)	Italian Duroc	IMF	[104]
		Italian Large White	ns	[104]
	g.5182A > T (intron 11)	Italian Duroc	BFT IMF	[104]
		Italian Large White	ns	[104]
PPARA	c.*636A > G (3′UTR)	Polish Landrace	BFT IMF	[105]
		Polish Large White	ns	[105]
PPARG	c.−1633C > T and c.−1572G > A haplotype (promoter)	Erhualian	IMF	[106]
PRKAG3	I199V	Large White	IMF	[107]
		Pietrain	IMF	[107]
		Duroc	ns	[107]
		Duroc × Landrace-Large White cross	ns	[108]
RYR1	c.1843C > T	Line 990	BFT AFW	[109]
SREBF1	c.1006T > C (exon 6) c.1033C > T (exon 6) c.1045C > T (exon 6) c.2911 + 130G > C (intron 17)	Polish Landrace	ns	[93]
	c.2911 + 320A > G (intron 17)	Polish Landrace	BFT, AFW	[93]

BFT, back fat thickness; *AFW*, abdominal fat weight; *IMF*, intramuscular fat; *ns*, nonsignificant.

expression of the *IRX3* gene, has recently been unraveled.[110] In contrast to studies on humans, there are no causative mutations detected in the pig *FTO* gene, which could be used as a universal marker of adipose tissue deposition. However, in recent years several reports have been focused on the relationship between *FTO* polymorphism and porcine fatness traits (Table 2). A g.276T > G SNP in intron 3 was associated with BFT both in Italian Duroc and commercial pig populations, while a weak association with IMF was observed in commercial pigs.[79] Moreover, it was also found that the frequency of the g.276G allele changed significantly during 20 years of selection toward leaner meat and lower BFT in Italian Large White pigs.[111] Another SNP, localized in the 5′flanking region of the *FTO* (g.−167T > G), was associated with fatness traits in Polish Landrace pigs, but not in the Polish Large White or a synthetic line 990.[81] A synonymous SNP (c.594C > G, alternatively described as FM244720: g.400C > G) also showed an association with some fatness traits, but the effects were again dependent on the population.[80] An extensive analysis of the *FTO* gene sequence in eight breeds revealed more than 30 polymorphisms arranged in 20 haplotypes and it was suggested that such a heterogeneity may hinder identification of variants predisposing to excessive lipid accumulation.[112] Interestingly, the *FTO* transcript level showed an inconsistent relationship with IMF in different breeds in a study performed by Tao et al.[113] It was reported that the *FTO* transcript level varied significantly between breeds, but it was not associated with IMF content in the muscle of various pig breeds. This may suggest that not the *FTO* itself, but an unknown regulatory element in this gene, being in a linkage disequilibrium with some SNPs in the *FTO* gene, exerts a significant phenotypic effect on fatness traits, similarly as it was described in humans by Smemo et al.[110]

The human *MC4R* gene, encoding a G protein-coupled transmembrane receptor involved in the control of appetite, energy homeostasis and body weight regulation, is highly polymorphic.[114] In the porcine ortholog numerous polymorphic variants have also been identified, but the results concerning their association with fatness traits are not conclusive.[115] During the last years several studies have been performed, supporting the hypothesis that the effect of the most extensively studied SNP (c.1426A > G, p.Asp298Asn) on fatness traits should be regarded as breed-specific (Table 2). For example, this polymorphism showed no significant effect on fatness traits in Duroc × Iberian crossbred pigs,[83] while in a study performed by Davoli et al.[84] a significant association with BFT in the Italian Large White was observed, whereas no effect was found in Italian Duroc pigs. The association with IMF in

commercial pigs, slaughtered at three local abattoirs, was reported by Rohrer et al.[86] and with BFT in Duroc pigs bred in Japan.[87] It was found that this SNP was the second most significant marker for BFT in Large White sows,[116] while the most significant was the g.3072G > A substitution in intron 3 of the *IGF2* gene, described earlier as a major marker for porcine meatiness and fatness by Van Laere et al.[117] The role of *MC4R* polymorphism was confirmed by a retrospective analysis of frequency changes in major genes, caused by 20-year long selection in the Italian Large White pig breed.[111] It was found that the frequency of the c.1426A allele increased significantly in that period.

Adipokines play a crucial role in energy homeostasis and thus the encoding genes are functional candidates for fat tissue accumulation traits. Polymorphisms of adipokine genes and their receptors have been extensively studied in the recent 5 years in relation to fatness phenotypes. The following ones were analyzed: leptin (*LEP*) and its receptor (*LEPR*), adiponectin (*ADIPOQ*), and resistin (*RETN*) (Table 2). Earlier studies of these genes were reviewed by Switonski et al.[16]

To date over 100 SNPs have been identified in the pig *LEP* gene.[90] Among them an effect of synonymous c.3469C > T substitution was the most extensively studied, but its association with fatness traits seems to be doubtful (Table 2). Other SNPs located in intron 2 of this gene were analyzed in the Iberian × Landrace cross and one of them (g.1387C > T) was associated with several traits, including BFT.[89] Two SNPs located in the 3′untranslated region (3′UTR), namely g.+747A > G and g.+846C > T, were tested for an association with fatness traits in the Polish Landrace breed, but only one of them, g.+846C > T substitution, showed a weak association with AFW (Table 2).[90]

The *LEPR* gene seems to be a more promising candidate gene. In a study of Munoz et al.[83] a missense SNP (c.2002C > T, p.Leu663Phe) showed significant effects on BFT and IMF (Table 2) and the T allele was associated with higher fatness. The same polymorphism, described by Perez-Montarelo et al.[89] as c.1987C > T, was analyzed in the Iberian × Landrace cross and the results for BFT and IMF were consistent with these reported by Munoz et al.[83] Further association analyses, performed in the Duroc breed, confirmed that the T allele has a strong effect on the increase of BFT.[87,91] The c.1987 T allele is fixed in the gene pool of a fat type Iberian breed and no haplotypic variability in this gene was observed when compared to other pig populations and wild boars from diverse European and Asian locations.[118] It is speculated that the *LEPR* gene region could have been under selection in

this breed, which resulted in increased fatness and other traits related to leptin resistance. The in silico analysis of RNA folding, performed by Ovilo et al.[119] to predict the effect of this SNP on mRNA, revealed an alteration in the RNA structure suggesting possible changes in the transcript stability. Concluding, the *LEPR* missense substitution c.2002C > T (c.1987C > T) appears to be a promising marker for economically important fatness traits in pigs. It should be mentioned here that based on the metaanalyses on the association of various *LEPR* gene variants with BMI and a GWAS, this gene should not be considered as an important candidate for excessive lipid accumulation in humans, except for rare monogenic forms of obesity.[74,120,121]

Polymorphisms of other adipokine genes have also been studied in relation to fat deposition in pigs, but there are fewer reports regarding their phenotypic effects. Two polymorphisms (c.−67G > A and 16 bp indel c.−106_−91delGCCAGGGGTGTGAGCC) in the promoter region of the *ADIPOQ* gene have been analyzed, but no association with fatness traits was found in the Polish Landrace and a synthetic line 990.[88] In the *RETN* gene a missense g.1473A > G SNP (p.Ala36Thr) showed a significant effect on IMF in the Landrace × Chinese–European crossbred population.[92] None of the aforementioned genes encoding adipokines and their receptors were among candidate genes showing significant associations with obesity in the large GWAS metaanalysis for BMI in humans.[74]

In studies focused on the identification of genetic markers for porcine fatness traits other genes have also been analyzed, including *ACACA*, *ACSL4*, *BMP5*, *CART*, *GNAS*, *HNF1A*, *ME1*, *MSTN*, *NAMPT*, *SCD*, *PCSK1*, *PPARA*, *PPARG*, *PRKAG3*, *RYR1*, and *SREBF1*. An overview of the association effects is presented in Table 2. Unfortunately, a majority of the identified polymorphic variants were not extensively analyzed in independent studies and quite often their effects were not consistent across breeds. Therefore their possible effect on fatness traits should be verified in further studies using various pig populations. Again, the aforementioned genes were not considered as candidates for predisposition to human obesity, listed by Locke et al.[74]

The association analyses quite often are accompanied by functional studies to unravel the possible molecular mechanism of the observed phenotypic effects. Such studies were undertaken when polymorphic variants were identified in regulatory regions (mainly promoter and 3′UTR) of the candidate genes. For example, the relevance of polymorphisms in the upstream regulatory regions of the *ADIPOQ*, *PPARG*, and *KDR* genes and 3′UTR

(near a putative miRNA target sequence) of the *PPARA* gene, were comprehensively analyzed using the in vitro luciferase assay and real time PCR.[88,105,106,122] A c.−1316A > G substitution in the *KDR* gene, encoding a vascular endothelial growth factor receptor, showed a significant effect on promoter activity (in vitro luciferase assay), the transcript level and was associated with IMF in the fat type Erhualian breed.[122] Similar results were obtained in the case of the *PPARG* gene, where two completely linked SNPs (c.−1633C > T and c.−1572G > A) affected the promoter activity and its transcript level was correlated with IMF in Erhualian pigs.[106] On the other hand, it was demonstrated that a 16 bp deletion in the 5′regulatory region (c.−106_−91delGCCAGGGGTGTGAGCC) of the *ADIPOQ* gene significantly changed the promoter activity in two cell lines, studied with the use of the luciferase assay approach, but no effect of the genotype was observed at the transcript level for any fatness traits.[88] Another in vitro study showed that c.*636A > G substitution in a putative microRNA target sequence in 3′UTR of the *PPARA* gene did not affect the interaction with miR-224, despite significant changes in the transcript level and an association with fatness traits in Polish Landrace pigs.[105] Such evidence coming from extensive molecular analyses facilitates verification of functionality of tested polymorphisms, as it was demonstrated in the case of the human *FTO* gene.[110]

Among candidate genes searched for association with pig fatness only *FTO* and *MC4R* showed a significant relationship with human obesity. Due to a strong selection to obtain lean and fast-growing pigs, a different set of genes may be involved in the regulation of lipid metabolism and adipose tissue accumulation in pigs in comparison to humans. Thus, knowledge concerning porcine genes with relevant functions in fat tissue accumulation may be useful in searching for novel candidate genes involved in hereditary predisposition to obesity in humans.

5.2 SNPs in Dogs

Importance of nongenetic factors predisposing dogs to obesity has been widely elucidated, while knowledge on DNA polymorphism associated with predisposition to this disease is poorly advanced.[17] Until now polymorphism was analyzed in the following candidate genes: *FTO*, *GPR120*, *INSIG2*, *IL6*, *MC3R*, *MC4R*, *PPARG*, *RETN*, and *TNF* (Table 3). Unfortunately, very few reports concerned their association with predisposition to obesity.

Table 3 Polymorphism and Association Studies of Candidate Genes for Dog Obesity.

Gene	Polymorphism (Localization)	Breed	Association Study	References
Genes, which human orthologs are associated with predisposition to obesity[74]				
FTO	23C > T Thr > Met (exon 1), 192A > T (intron 1), 223T > C (intron 1), 378053G > A (3´flanking), 378284T > C (3´flanking), 378318G > C (3´flanking)	Various breeds	Not tested	[123]
MC4R	c.637G > T p.Val213Phe (exon 1), c. 777 T > C (exon 1), c.*33C > G (3′UTR)	Various breeds	Not tested	[124]
	c.637G > T p.Val213Phe (exon 1), c. 777 T > C (exon 1), c.868C > T (exon 1), c.*33C > G (3′UTR)	Golden Retriever	No association with morphological measures	[125]
	A420 C (Asp101Thr) (exon 1)	Beagle	Significant association with body weight	[126]
	C895 T (exon 1)	Beagle	Significant association with female body weight	[126]
Other candidate genes				
GPR120	c.252C > G, c.282C > G, c.287T > G p.Leu96Arg, c.307G > A p.Ala103Thr, c.446G > C p. Gly149Ala, c.595C > A p.Pro199Thr, c.702A > G, c.726G > A, c.984T > C	Various breeds	Frequency of variants at c.595C > A significantly differed between lean and overweight/obese dogs	[127]

(*Continued*)

Table 3 Polymorphism and Association Studies of Candidate Genes for Dog Obesity.—cont'd.

Gene	Polymorphism (Localization)	Breed	Association Study	References
INSIG2	−91G > A (5´flanking), −1C > T (5´flanking), 40C > A Arg > Ser (exon 1) 1483A > T (intron 1) 1637C > T (intron 1) 2169G > A (intron 1) 10820T > A (intron 4)	Various breeds	Not tested	[123]
MC3R	c.−90T(11_13) (5′ flanking region), c.142C > T (exon 1)	Various breeds	Not tested	[128]
PPARG	C1362 T (exon 7)	Mongrels, Miniature Dachshund	Not tested	[129]
TNF	c.−40A > C (5′UTR), c.249C > T (exon 1), c.548A > T p. Glu183Val (exon 4), c.627C > T (exon 4), c.186 + 16A > G (intron 1), c.186 + 174GAAT$_{[N]}$ (intron 1), c.186 + 211C > T (intron 1), c.187 − 47T > C (intron 1), c.233 − 54T > C (intron 2), c.233 + 14G > A (intron 3), c.233 + 17G > T (intron 3), c.233 + 108A > G (intron 3), c.*107G > A (3′UTR)	Various breeds and mongrels	Frequency of variants at c.−40A > C and c.233 + 14G > A significantly differed between lean, overweight and obese Labradors. Other polymorphisms not analyzed.	[130]

IL6	c.102T > C (exon 2), c.572G > A (exon 5), c.309 + 215T > C (intron 3), c.*283G > A (3′UTR)	Various breeds and mongrels	No significant differences of allele frequencies at c.309 + 215T > C between lean, overweight and obese dogs of various breeds. Other SNPs not analyzed.	[130]
RETN	c.19C > T (p.Leu7Phe), c.75G > A, c.115 + 29G > C, c.115 + 143T > G, c.116 − 179G > A, c.141C > T, c.194–69T > A, c.236C > G	Various breeds and mongrels	No significant differences of allele frequencies at c.19C > T, c.75G > A and c.194 − 69T > A between lean, overweight and obese dogs of various breeds. Other SNPs not analyzed.	[130]

The *MC4R* was the most frequently investigated gene. Altogether six polymorphic variants, including two missense ones, have been reported (Table 3). In comparison with the human *MC4R* gene,[114] the canine counterpart seems to be much less polymorphic, however, it should be pointed out that this suggestion may be biased due to a much smaller number of the analyzed dogs, when compared with studies in humans. A missense A420 C (Asp101Thr) substitution showed an effect on body weight in a small cohort of Beagles (males and females), while a silent C895 T SNP was associated with body weight of females only.[126] Other SNPs in this gene (missense c.637G > T and two silent substitutions: c. 777 T> C and c.868C > T, and c.⋆33C > G in 3′UTR) showed no association with morphological measures, including weight and body index score [weight/(length × height)] of Golden Retrievers.[125]

Among numerous polymorphisms found in the canine *MC3R*, *FTO*, *INSIG2*, and *PPARG* genes there were two nonsynonymous SNPs. Unfortunately, none of the identified variants was tested for an association with canine obesity.

Nine SNPs were detected in the coding sequence of the *GPR120* (G-protein-coupled receptor 120) gene, including four missense substitutions (Table 3). The frequencies of a nonsynonymous substitution (c.595C > A, p. Pro199Thr) were analyzed across 20 breeds and a group of mongrel dogs with different BCSs, with a significant difference found between BCS3 (lean) and BCS4/BCS5 (overweight/obese) dogs.[127]

Recently three adipokine genes (*TNF*, *RETN*, and *IL6*) were studied in lean, overweight, and obese dogs.[130] In a screen comprising 17 breeds and mongrels, a total of 25 polymorphisms were found in these genes, including nonsynonymous substitutions in *TNF* and *RETN*. Distribution of six SNPs (two in *TNF*, one in *IL6*, and three in *RETN*) was analyzed in dogs in relation to obesity (Table 3). It was found that frequencies of two SNPs in noncoding regions, c.−40A > C in 5′UTR and c.233 + 14G > A in intron 3 of the *TNF* showed significant differences in Labrador dogs with different BCS.

It is rather surprising that genetic studies of canine obesity, an emerging and serious health problem, are so scarce, even though the dog genome sequence has been available for a decade.[66] It seems that the *MC4R*, *GPR120*, and *TNF* genes are potential markers for hereditary predisposition to obesity at least in some breeds (Labrador Retriever and Beagle). It is expected that in the near future more genetic studies will be focused on the association of polymorphic variants of candidate genes, selected on the

basis of knowledge concerning genetic markers predisposing humans to obesity (eg, *FTO*, *MC4R*, *TMEM18*, *GNPDA2*, *GABRG1*, *SEC16B*, etc.).

5.3 CNVs in Pigs

CNV is a class of structural variation of the genome and is defined as a DNA segment (from 1 kb up to several Mb) that is present at a variable copy number when compared to a reference genome.[131] The recent human CNV map revealed that CNVs represent about 4.8–9.5% of the genome.[132] It was shown that this type of genomic variations is responsible for human diseases.[133] There are several reports concerning an association of CNVs with human obesity. In these CNV regions several candidate genes were indicated, for example, *SH2B1* on chromosome HSA16,[134,135] *PPYR1* on HSA10,[136] and *AMY1* on HSA1.[137]

Many efforts have been made to detect CNVs in the pig genome with the use of different technologies, such as the CGH array, SNP genotyping array and genome resequencing. The latest study on the characterization of CNVs in the pig genome was performed with the use of a custom-designed 1 M CGH array in 12 pig samples from diverse pig breeds, including the Asian wild boar, Chinese indigenous, and European commercial breeds.[138] The authors identified 758 CNV regions (CNVRs), covering 47.43 Mb of the pig genome sequence, which corresponds to 1.69% of the genome. More than 1200 genes were completely or partially overlapped with the identified CNVRs. So far, only a few attempts were made to identify CNVs in pigs in relation to production traits, including fatness. Trait-related CNVs were studied in 18 diverse pig populations with the use of the porcine 60k SNP microarray (Porcine SNP60 BeadChip) and 538 CNVs were identified in a White Duroc × Erhualian F2 population.[139] Integrating previous QTL mapping data with the detected CNV regions facilitated the identification of seven candidate genes (*ANP32B*, *BSCL2*, *LTBP3*, *GDF3*, *GYS1*, *KIT*, and *CAV1*) for several traits, including fatness parameters: BFT, AFW, and IMF. In 2013 Fowler et al.[140] using the same Porcine SNP60 BeadChip compared fat and lean samples derived from 3 commercial breeds (the Large White, Duroc, and a white Pietrain composite line) and identified 12 CNV regions, which harbored 4 functional candidate genes (*MCHR1*, *PPARA*, *SLC5A1*, and *SLC5A4*) for fatness traits.

Analysis of CNVs specific to indigenous pig breeds may be useful in the identification of the genetic background responsible for phenotypic variation, including fatness. Resequencing of genomes for three variants of the

Mangalica breed (Blond, Red, and Swallow-belly) led to the identification of approx. 1000 CNV gains (CNV losses were not analyzed due to a low coverage of sequencing).[73] However, no CNVs associated with fatness traits were detected. In another study, carried out in Chinese breeds, it was shown that Meishan pigs have a high number of copies of *AADAT* and *ZNF622* genes,[141] which may be related with a lower growth rate, but again no CNVs for fatness traits were identified. A comparison of CNVs in four Chinese normal size breeds (Luchuan, Tongcheng and Laiwu pigs) and two minipig breeds (Bama and Wuzhishan) did not identify CNVs influencing growth of these breeds as well as fatness.[142]

Searching for CNVs in commercial, meat-type breeds, were also carried out. Such a study, concerning Italian Large White pigs, was reported by Schiavo et al.[143] They analyzed two groups of animals (approx. 150 animals each) with extreme BFT values using the 60k SNP microarray (Illumina Porcine SNP60 BeadChip). Sixteen CNV regions (CNVR) located on six autosomes (SSC8, 11, 12, 13, 14, and 15) showed an association with BFT; however, this result appeared to be nonsignificant after the Bonferroni correction. Among the detected CNVRs, 14 represented low-frequency CNV events, while 9 high-frequent were observed in fatter pigs. One of the CNVRs encompassed the *ZPLD1* gene, which was also found in the human CNV associated with childhood obesity.[144] Thus, further studies of this chromosomal region are advisable. The lack of CNVs significantly associated with BFT may be explained by the power of the experiment, since the identification of low frequent markers may require a larger cohort of investigated animals. It is also possible that this type of polymorphism may have a limited impact on adiposity in pigs. A previous analysis of CNVs in both domestic and wild pigs,[145] which allowed to identify 3118 CNVRs, revealed that CNVRs reflect demographic history rather than phenotypic diversity.

5.4 CNVs in Dogs

So far, there have been no reports on the discovery of CNVs associated with obesity in dogs. However, there is an extensive study on dog domestication involving genotyping 1611 dog CNVs in 23 wolf-like canids.[146] This approach facilitated the identification of 25 CNVRs that showed the largest differentiation between dogs and wild canids. Within CNV regions there are 12 candidate genes, which function is related with growth and neurological function. One of the promising candidate genes for body weight is the *PDE4D* gene. The region containing this gene presented gain in all wild

canids, whereas losses were observed in a majority of the studied dogs (Boxer, Beagle, and Basenji). It is known from studies on mice that PDE4D-deficient young mice exhibited delayed growth and a decreased level of circulating IGF-I.[147] The second gene identified in the study of Ramirez et al.[146] was *CRTC3*, for which a higher copy number was observed in dogs than in gray wolves. Interestingly, variants of this gene have been associated with adiposity in humans.[148,149]

An interesting discovery concerns copy numbers of the canine amylase gene. It should be mentioned that most mammals, including the human, express amylase in saliva, but dogs produce amylase in the pancreas only. The gene encoding the pancreatic type of amylase (*AMY2B*) is found only in two copies in wolves, while in dogs the copy number ranges from 4 to 30.[150] The increase in the number of copies is associated with a high activity of this enzyme and it is believed that it allows more efficient digestion of starch. A further study of Arendt et al.[151] revealed that copy numbers of the *AMY2B* vary between breeds, as well as individuals. Since amylase activity may predispose to obesity and diabetes mellitus in humans,[152] the *AMY2B* copy number and susceptibility to diabetes mellitus in dogs was analyzed, but no association was found.[151] However, the authors indicated that a larger collection of cases should be evaluated in future studies. Studies on pigs have shown that conversely to humans and dogs, this species has a universally high number of copies—from 8 to 21 copies of amylase genes (*AMY1*, *AMY2A*, *AMY2B*).[145]

6. CONCLUSIONS AND PERSPECTIVES

The pig and the dog are known as important large animal models for human diseases, including obesity. However, an excessive accumulation of adipose tissue has different significance in these model species. In the pig it is considered as an important production trait and thus extensive studies on its genetic background have been carried out. In the dog obesity is an emerging health problem, but until now genetic studies of this disease are not advanced.

A comparison of candidate gene sets, which polymorphisms are associated with adipose tissue accumulation, in the human (97 loci listed by Locke et al.[74]) and pigs (22 loci presented in Table 2) revealed distinct differences. Only two porcine genes, associated with adiposity (*FTO* and *MC4R*), were indicated in the human and the pig, while others (eg, *LEP*, *LEPR*, *SCD*, etc.) were specific to pigs only. It may be caused by different factors, including the

size of the studied cohorts (very large in humans, especially when metaanalyses were carried out), number of the studied traits—usually body mass index (BMI) in humans and several traits in pigs (BFT at several points, ABW and IMF), and different breeding strategies, which modified the gene pool of pig breeds representing a specific type (fat, meat, autochthonous, experimental strains—minipigs, etc.).

Phenotypic evaluation of dog adiposity is commonly based on a subjective BCS scale. Unfortunately, it may bias the association analysis. On the other hand, the dog seems to be a very suitable model for human obesity because both species share the same environment, including diet and physical activity and a quite long life span of dogs. Moreover, it is important to point out that there are dog breeds predisposed to obesity (eg, Labrador and Golden Retrievers, the Cocker Spaniel, Beagle, etc.), as well as those characterized by limited depots of adipose tissue (eg, the Greyhound, Whippet, etc.).

Clearly, in the near future more studies will be carried out on the genetic background of obesity, especially in dogs. Since the impact of common polymorphisms (SNPs and CNVs) on human obesity is limited, it is suggested to focus on epigenetic markers in further studies on model organisms, including the pig and the dog.

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