
Applications of Systems Genetics and Biology for Obesity Using Pig Models

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Abstract

In many biomedical research areas, animals have been used as a model to increase the understanding of molecular mechanisms involved in human diseases. One of those areas is human obesity, where porcine models are increasingly used. The pig shows genetic and physiological features that are very similar to humans and have shown to be an excellent model for human obesity. Using pig populations, many genetic studies have been performed to unravel the genetic architecture of human obesity. Most of them are pinpointing toward single genes, but more and more studies focus on a systems genetics approach, a branch of systems biology. In this chapter, we will describe the state of the art of genetic studies on human obesity, using pig populations. We will describe the features of using the pig as a model for human obesity and briefly discuss the genetics of obesity, and we will focus on systems genetic research performed using pigs with their contribution to human obesity research.

1 The Pig as a Model for Human Obesity

Throughout the history of biomedical research, animals have been extensively used as a model for human diseases. Animal models have several advantages with respect to costs, ethical potential, and measurement of phenotypic characteristics. The use of animal models in biomedical research has been previously described in depth (Hau 2008), showing that the choice of animal model in biomedical research is

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highly dependent on the genetic, physiological, and/or psychological features of both animal and disease under study. For human obesity, rodents are a commonly used animal model, but because of major anatomical and physiological differences, the translational efforts to human medical science have been limited (Haupt et al. 1979; Spurlock and Gabler 2008). To overcome those major differences, the pig (*Sus scrofa*) has successfully been used as a model for human obesity.

The digestive tract is one of the key organs in obesity research (Halsted 1999) and, therefore, needs to be considered when choosing an animal model for human obesity. Zooming in on the anatomy of the digestive tract of pigs, it can be shown that it is very similar to that of humans. Both species are omnivorous, and their digestive tract consists of the esophagus, stomach, small intestine (consisting of duodenum, jejunum, and ileum), and large intestine (consisting of cecum, colon, rectum, and anus). Furthermore, the digestive tract has proportionally the same size: the stomach has a capacity of 6–8 l in the pig compared to 2–4 l in humans (Curtus and Barnes 1994), the small intestine is approximately 18 m long in the pig (Razmaite et al. 2009) compared to 7 m in humans (Gray 1918), and the large intestine is approximately 6 m in the pig (Razmaite et al. 2009) compared to 1.5 m in humans.

Also genetically, the pig is very similar to humans. Recently (2013), the pig genome was assembled and analyzed (Groenen et al. 2012), resulting in the annotation of protein-coding genes and gene transcripts, with numbers comparable to the human genome (Table 1).

Based on the pig's genetic background, Groenen et al. (2012) also showed the potential of the pig as a biomedical model. For example, they detected that at 112 positions, the amino acid sequences were equal to the human orthologs that were implicated in human disease. Moreover, several studies have used the pig as a biomedical model, regarding, for example, heart physiology, brain, gut physiology and nutrition, biomechanical models, respiratory function, and infectious disease models (Lunney 2007; Michael Swindle and Smith 2008). Here, we will focus on the use of the pig as a model for human obesity and its application in systems biology research.

Already in 1979, the use of the pig as a biomedical model to study human obesity was reviewed (Haupt et al. 1979). Several similarities between the pig and the humans were discussed with respect to obesity-related phenotypes. For example, both in humans and in pigs, fat is mainly stored in subcutaneous adipose tissue, and fat cell size and number are similar (Gurr et al. 1977). High-density lipid protein

Table 1 Overview genome of the pig and human

	Pig	Human
No. of chromosomes	19 pairs	23 pairs
No. of base pairs	2,596,639,456	3,272,480,989
Protein coding genes	21,640	20,345
Pseudogenes	380	14,206
ncRNAs	2,965	22,883
Gene transcripts	26,487	196,520

(HDL) is also structurally and compositionally similar (Davis et al. 1974). Importantly, as in humans, there is no indication that obesity in pigs is caused by a single locus or gene, but it is most likely caused by several loci or homozygous recessive genotypes for obesity. Genetically, there is, however, a difference in various pig breeds: a number of breeds show a strong propensity for obesity (e.g., Ossabaw pig and Göttingen minipig), whereas others have been bred for centuries for their lean meat content (e.g., Yorkshire and Duroc).

As mentioned, the pig as a biomedical model has major advantages in comparison with human studies with respect to the measurement of phenotypic characteristics. Because of costs and ethical reasons, it is easier to measure a wide range of phenotypes under controlled experimental conditions, and after the study period, the animal was slaughtered and samples from different tissues and cells were collected. Also during the study period, deep phenotyping can be obtained using dual-energy X-ray absorptiometry (DXA) scanning. DXA has shown its potential in human obesity studies because of the precise measurement of the body composition with respect to fat mass. Several studies have shown the potential of estimating the fat mass percentage of pigs using DXA scanning. For example, it was shown that the percentage of body fat measured by DXA was not significantly different from estimation by chemical analysis, but more extensive calibration may be needed for total body analysis (Mitchell et al. 1996), which was similarly shown in small pigs (Mitchell et al. 1998). Likewise, a study using production pigs (Large White $\times$ Landrace pigs) showed a high accuracy of determining body composition using DXA scanning (Suster et al. 2003).

One of the well-known pig breeds in relation to obesity is the Göttingen Minipig. This minipig is bred for its small size and ease in handling, which is one of its main advantages in an experimental setting (Johansen et al. 2001). It has been shown that this breed becomes severely obese when fed a high-fat, high-energy diet, and their glycemic control is similar to what has been observed in humans (Johansen et al. 2001). For example, pigs fed the high-fat, high-energy diet had a fat content of $15.2\% \pm 0.7\%$ vs. $10.0\% \pm 1.2\%$ in the low-fat, low-energy diet. However, dissimilarities were also observed with the high-fat, high-energy diet: the obese pig showed an increase in triglycerides and HDL cholesterol concentration, whereas in the obese human, triglyceride levels were increased but HDL cholesterol values were decreased. Surprisingly, the Göttingen Minipig also develops obesity on a normal, ad libitum diet and, therefore, is classified to be prone to obesity (Bollen et al. 2005). Another breed with high potential in obesity research is the Ossabaw pig, another miniature pig breed. They possess a thrifty genotype, similar to humans, which results in the ability to store large amounts of fat during feasting and consequently survive periods of famine (Dyson et al. 2006). Studies have shown their excellent potential as a model for obesity and the progression to type 2 diabetes and other implications of obesity (e.g., coronary artery disease). Extensive discussions on type and use of many breeds of minipigs in biomedical research and sources to procure them for research can be found in the book *The Minipig in Biomedical Research* (McAnulty et al. 2011).

In contrast to the obese pigs, production pigs (e.g., Duroc and Yorkshire) have been bred for centuries for less fat or for their lean meat content to live up to the standards for human consumption, leading to a pig breed that is genetically predisposed for leanness. Although those animals are less valuable in an experimental setting because of their size, the normal production setting has great potential. In research, e.g., performed by animal breeding industries, a large amount of data are collected to breed animals that are growing fast and lean, with a high feed efficiency. These data have vast opportunities to be related to human obesity studies, gaining knowledge about the genetic architecture of, for example, eating behavior and development of lean/fat content.

2 The Complexity of Human Obesity in a Nutshell

Obesity is the excessive accumulation of body adipose tissue, commonly the result of a chronic imbalance between energy intake and expenditure (Galgani and Ravussin 2010). Obesity is mostly the result of both environmental and genetic factors and interactions among and within them (multi-factorial) (Bougnères 2002). Worldwide, the prevalence of obesity has been growing exponentially over the last decades (World Health Organization 2012), which may be largely due to the increased availability of energy-rich foods and reduced need for physical activity (O'Rahilly and Farooqi 2006). However, it is also known that there is a large genetic component: quantitative genetic studies have estimated the heritability of obesity to be between 40% and 70% (Speliotes et al. 2010). The regulation of energy balance (homeostasis) is a very important aspect of human obesity. Many different tissues, biological processes, and hormones are involved, whereby genes also play a major role. For example, energy/food intake is strongly associated with appetite and satiety. Those states are mainly regulated by the central nervous system with several involved organs and hormones. One of these hormones is ghrelin, an appetite-stimulatory signal secreted by the stomach (Wren et al. 2001). On the other side, leptin is called the satiety hormone, released by adipose tissue and functioning through its receptor in the hypothalamus, leading to a reduction of food intake and increase of energy expenditure (Friedman 2002).

Obesity is also very closely related to the human immune system because of the variety of functions of adipose tissue, i.e., endocrine, inflammatory and metabolic functions (Heber 2010). White adipose tissue mainly consists of adipocytes that store energy as fat. Adipocytes secrete a large number of adipokines (also called adipocytokines) with important roles in energy homeostasis (Fantuzzi 2005). The most abundant ones are leptin (function mentioned previously) and adiponectin (Tilg and Moschen 2006). Adiponectin is involved in insulin sensitivity and has anti-inflammatory and anti-atherogenic properties (Diez and Iglesias 2003). Besides adipocytes, several immune cells are present in adipose tissue (Ferrante 2013), of which the most abundant are macrophages with an important role in phagocytosis. Among others, neutrophils (critical for the first immune response) and mast cells

(several immune functions, e.g., role in allergy) are strongly increased in individuals with obesity (Elgazar-Carmon et al. 2008; Liu et al. 2009).

The complexity of obesity as well as, for example, the relationship of adipose tissue with many different hormones and cells results in its association with several other (complex) diseases, like type 2 diabetes, cardiovascular problems, and several types of cancer. For instance, decreased insulin sensitivity (leading to type 2 diabetes) is a consequence of the chronic, low-grade inflammation state caused by the increased level of immune cells because of the high degree of adipose cells (Xu et al. 2003; Shoelson et al. 2007). Insulin has an important function in regulating the uptake of glucose, originating from carbohydrates in nutrition. Because of food intake, the blood glucose levels rise, and insulin makes sure that the glucose is transported to the cells and that cells take up glucose so it can be used as an energy source. In the case of insulin sensitivity, there is not enough insulin, resulting in a limited uptake of glucose (type 2 diabetes). As the human body is dependent on an adequate glucose supply, the disturbed glucose uptake has major consequences for the human body, such as heart and vascular problems, diabetic retinopathy (affected eyesight), and kidney failure. Furthermore, insulin also has an anti-inflammatory effect, again relating obesity to the immune system.

The complexity of obesity becomes clear by looking into all the associated tissues, cells, hormones, and biological mechanisms and thereby affects longevity and quality of human life. Its consequences for human health and subsequent financial burden on the society increase the need for a better understanding of the biological and genetic background of obesity.

3 Single Gene Studies in Obesity: What Do We Know So Far?

For several years, genomewide association studies (GWAS) have been very important in the detection of genes associated with diseases. Since 2007, GWAS have been published regarding obesity. Most commonly, the body mass index (BMI) was used as a measure of obesity. The first GWAS performed on BMI, using 4741 individuals and 362,129 SNPs, led to the detection of the fat mass and obesity-associated (*FTO*) gene (Scuteri et al. 2007). Approximately 8 years later, the latest GWAS performed on BMI, composed of 339,224 individuals and approximately 2.5 million SNPs (GIANT consortium), detected 97 obesity-related loci (Locke et al. 2015). Although this is a huge increase in detected loci/genes associated with obesity, results thus far are disappointing, as they only explain approximately 2.7% of the BMI variation. Over the years and with new findings, the understanding of biological mechanisms behind obesity has also changed. Where *FTO* was discovered as an actual fat mass gene (related to feed intake), pathway analysis of the 97 obesity-related loci discovered by Locke et al. (2015) is pointing toward a major role for the central nervous system.

Furthermore, several other phenotypes have been used to detect obesity-related genes. For example, it has been shown that central obesity has more negative health

consequences than general obesity. As a consequence, the waist-to-hip ratio (WHR) might be more informative than the BMI (Vazquez et al. 2007; Molarius and Seidell 1998). Recently, 49 loci related to WHR were detected using the GIANT consortium database, consisting of genes that were highly enriched in adipocyte-related tissues (Shungin et al. 2015). Likewise, as with GWAS on BMI, the variance explained by those loci is very low: only approximately 1.4% of the variance was explained.

Many more studies have been performed to try elucidating the genetic background of obesity, to gain understanding of the biological mechanisms of this complex phenotype. As mentioned, the use of animal models in biomedical research has some outstanding advantages, such as costs and ethical potential. Several research groups have made use of those potentials and investigated obesity using the pig as a biomedical model, either using data sets coming from the pig industry or by using an experimental animal model, both having their own (dis)advantages (Fig. 1).

4 Human Obesity Genes Present in Pigs

The first gene that has been directly associated with obesity in humans is the *FTO* gene. This gene has also been related to obesity-related traits in pigs by several studies. In 2007, a study focused on the alleles of the *FTO* gene and studied the relationship of this gene in seven pig breeds with several measured traits (Fontanesi et al. 2009). They showed, and reconfirmed, that *FTO* was significantly associated with obesity-related phenotypes in Duroc pigs, for example, intramuscular fat content (Fontanesi et al. 2010). Also in an ISU Berkshire $\times$ Yorkshire population, this gene showed significant association with average daily gain and total lipid percentage in muscle (Fan et al. 2009). Furthermore, an expression study showed elevated levels of the *FTO* gene brain tissues, with significantly higher levels in the cerebellum compared with the cortex of pigs fed a high-cholesterol diet (Madsen et al. 2009).

Another well-known human obesity gene is *MC4R*. The gene is active in the hypothalamic leptin–melanocortin signaling pathway and has been associated with suppression of food intake (Santini et al. 2009). Also in pigs, the gene has been associated with several obesity-related traits. In Italian Duroc and Italian Large White pigs, it has been associated with daily gain, feed conversion ratio, and ham weight (Davoli et al. 2012). Another study using Large White showed the association of *MC4R* with backfat depth, average daily gain, and daily feed intake (Houston et al. 2004).

Another study that showed the presence of human obesity genes in pigs was performed at Poznan University of Life Sciences (Poland). They localized seven previously mapped (*INSIG2*, *LIPIN1*, *PLIN*, *NAMPT*, *ADIPOQ*, *UCP2*, and *UCP3*) and six novel (*NR3C1*, *GNB3*, *ADRB1*, *ADRB2*, *ADRB3*, and *UCP1*) candidate genes for human obesity in the pig genome (Nowacka-Woszek et al. 2008). All genes could be localized on one of the pig chromosomes, and several of them were located within a known quantitative trait loci (QTL) for fatness traits in pigs.

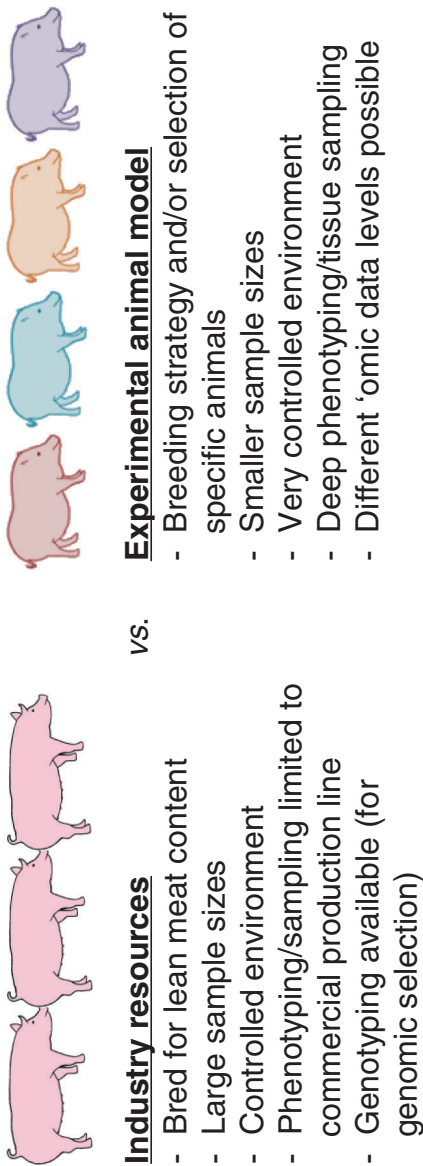


Fig. 1 Overview of potential use of pigs for human obesity research; industry versus experimental animal model

Another expression-based study also showed the presence of *LEP*, *LEPR*, *NEGR1*, and *ADIPOQ* together with *FTO* and *MC4R* in production pigs and in Göttingen Minipigs (Cirera et al. 2014).

5 Studying the Genetics of Fatness Traits in Pigs: Input from the Industry

Fat-related traits in pigs have been studied intensively for the pig industry because of their commercial effect. Production pigs have been bred for their lean meat content, which means that it was commercially interesting to detect biomarkers for fatness. Although many of these studies have never been related to human obesity, it can be proposed that those genes might be important in a human context. For example, a QTL study for backfat thickness and intramuscular fat content in an experimental cross between Meishan and Dutch Large White and Landrace lines detected several QTLs on several chromosomes. Comparative mapping of the results showed human homologues for those QTLs, for example, tumor necrosis factor α (de Koning et al. 1999). Furthermore, in a QTL study using a three-generation experimental cross between Meishan and Large White pig breeds, seven chromosomal regions were detected as genomewide significant for fatness traits. One of the detected QTLs was close to the insulin-like growth factor 2 locus (Bidanel et al. 2001). A GWAS performed on 669 Duroc pigs across generations resulted in the detection of a region associated with backfat thickness. In this region, six genes were identified (*PDE4B*, *LEPR*, *LEPROT*, *DNAJC6*, *AK3L1*, and *JAK1*) of which both *LEPR* and *PDE4B* have previously been associated with backfat-related traits and obesity.

A GWAS study of 820 commercial sows (Large White and Large White $\times$ Landrace cross) identified several regions associated with backfat, including the genes *MC4R*, *ATP6V1H*, *OPRK1*, *LDHD*, *CHCHD3*, and *ATP2B3* (Fan et al. 2011). Of those, *MC4R*, *ATP6V1H*, and *CHCHD3* have been previously associated with obesity in human or other animal studies (Hwang et al. 2010; Lubrano-Berthelier et al. 2003; Walewski et al. 2010). Another GWAS using 651 Duroc samples (three generations) detected a region associated with backfat thickness containing six genes: *PDE4B*, *LEPR*, *LEPROT*, *DNAJC6*, *AK3L1*, and *JAK1* (Okumura et al. 2013). Several of those have been previously associated with human obesity, such as the previously discussed *LEPR*.

In addition to GWAS performed on backfat and other fat-related traits, another study focused on feeding behavior in production pigs (Duroc boars), which might be an important trait with respect to feed efficiency (Do et al. 2013). However, findings in this study could also be valuable for human research because of its impact on eating behavior and subsequent development of obesity. Among the genes that were discovered were *ENPP1*, *HCRT*, and *MTTP*.

Another study focused on the sequencing of the porcine genome using RNA sequencing and found a gene associated with cholesterol and triglyceride levels: *CES1* (Chen et al. 2011). Interestingly, a human study has also shown that the

expression of *CESI* is upregulated in obese subjects (Marrades et al. 2010), whereas in mice, it was shown that *CESI* has an important role in lipid homeostasis (Xu et al. 2014).

6 Porcine Models for Human Obesity

As described, the pig is an excellent model for human obesity with huge potential. Many researchers have taken this opportunity to study human obesity, using different porcine models. In this section, we will describe several of those studies in order to gain insight in to the knowledge gained about human obesity using porcine models.

One of the ways in which pigs are used as a model for human obesity is by inducing obesity with a high-fat diet. Using this approach, Li et al. (2011) created a porcine model of 20 crossbred boars, which were fed a corn-soy basal diet or a diet with lard for 180 days. There were significant differences between the high-fat diet pigs and control pigs with respect to, for example, body weight, backfat thickness, and abdominal fat. They detected 852 genes (387 upregulated and 465 downregulated) that showed differential expression between the obese pigs and control pigs. Upregulated genes were mainly associated with metabolic process, immune response, translation, and cell cycle, whereas downregulated genes were mainly involved in regulation of transcription, RNA splicing, and transcription.

Porcine models can also be obtained by intercrossing pig breeds, creating a population consisting of different generations that is advantageous for genetic studies. An excellent example is the UNIK pig resource population (University of Copenhagen, Denmark), which is an F2 pig population created by intercrossing Göttingen Minipig boars with Duroc and Yorkshire sows (563 pigs). As described earlier, the Göttingen Minipig and production pigs (i.e., Duroc and Yorkshire) are very distinct in some obesity-related features, e.g., body size and body fat content. By creating an F2 intercross, the F2 animals will show a wide range of values for each distinct phenotype. This large variation in obesity-related phenotypes was also obvious in the UNIK pig resource population, shown by genetic parameter calculations. The high coefficients of variation (15–42%) and moderate to high heritabilities (0.22–0.81) for obesity and obesity-related traits showed that the population was highly divergent for obesity traits (Kogelman et al. 2013). Furthermore, genetic correlations between obesity-related traits revealed more of the genetic architecture in the population. For example, weight and lean mass estimated by DXA scanning were highly correlated (0.56–0.97), and fat-related traits were strongly correlated with glucose levels (0.35–0.74). The UNIK resource population thereby proved its potential for further genetic investigations using, for example, genomics and transcriptomics.

To detect genomewide associations for obesity-related phenotypes in the UNIK pig resource population, a combined linkage disequilibrium-linkage analysis was performed (Pant et al. 2015). This resulted in the detection of 229 QTLs that were subsequently used for comparative analysis with the human genome. Many different

genes were identified for obesity-related phenotypes, such as BMI (e.g., *SMAD6* and *PAX5*), fasting glucose levels (*STIM1*), and cholesterol levels (e.g., *STRADA*). Another study within the UNIK pig resource population focused on obesity-specific microRNA expression in subcutaneous adipose tissue (Mentzel et al. 2015). In total, two differentially expressed microRNAs were discovered and validated using qPCR: mir-9 and mir-124a. Both microRNAs have been previously associated with obesity-related phenotypes in human and/or mouse studies.

7 Systems Genetics Analyses of Obesity Using a Porcine Model

As previously described, obesity is a complex disease involving many different genes, pathways, tissues, and organs. With respect to complex diseases, systems genetics approaches can be very useful to unravel the involved molecular mechanisms. One of those systems genetics approaches is expression quantitative trait loci (eQTL) mapping, whereby genomic and transcriptomic data are integrated. Generally, this leads to the detection of both *cis*- and *trans*-eQTLs, where SNPs of a *cis*-eQTLs are located near the affected transcript and SNPs of a *trans*-eQTL are mapped further away from the affected transcript or even on a different chromosome.

In 2011, a group at the Leibniz Institute for Farm Animal Biology (Germany) performed the first eQTL mapping in a porcine model to analyze obesity-related traits (Ponsuksili et al. 2011). A total of 150 pigs (Pietrain $\times$ (German Large White $\times$ German Landrace)) were genotyped and expression-profiled using microarray platform. First, they detected genes that were correlated with fat traits and showed that positively correlated genes were mainly related to metabolism of various macromolecules and nutrients. On the other hand, negatively correlated genes were related to dynamic cellular processes. Second, the eQTL mapping was performed, leading to the detection of 448 *cis*-eQTLs and 3297 *trans*-eQTLs. Next, pathway analysis and network generation were performed on the detected genes within the eQTLs. Constructed networks within *cis*- and *trans*-eQTLs resulted both in detection of pathways related to lipid metabolism. Overall, they detected several genes that were previously detected in human and/or mouse studies (e.g., *PRDX6*, *PLIN4*, and *FOXO1*), but also detected novel candidate genes (e.g., *SLC12A1*).

In the same year, another eQTL mapping study on an F2 pig population was performed (Steibel et al. 2011). Although this F2 population was not created as a model for human obesity, they did find several eQTLs that were part of a network associated with lipid metabolism. In the networks associated with lipid metabolism, many genes were present, such as *AKR7A2*, *CASP7*, and *CYP4F2*. Results of this study were proposed as novel candidate genes for pig production traits, but a subset of them could potentially be translated to human obesity.

Another way of data integration was performed by integration of heritability estimates with comparative genomics strategy to identify causal genetic factors (Kim et al. 2012). First, they showed that the human chromosome 2 is mostly

syntenic to chromosome 3 and chromosome 15 of the pig. Second, heritabilities for backfat thickness in the pig and subscapular skinfold thickness in humans were estimated, and a correlation of 0.479 was detected. In both the pig and human populations, the human chromosome 2 was detected as the most important chromosome based on the percentage of heritability explained and was therefore further investigated. Several genes were suggested as being candidate genes for human obesity, namely, *MRPL33*, *PARD3B*, *ERBB4*, *STK39*, and *ZNF385B*.

In the previously mentioned UNIK pig resource population, several systems genetics approaches were also conducted. Using the 60K genotype data, genetic networks were constructed using the weighted interaction SNP hub (WISH) method (Kogelman et al. 2014b). The WISH method detects interactions between SNPs based on their genotype correlations and based on the epistatic interaction effect between SNP pairs (Kogelman and Kadarmideen 2014). First, a GWAS was performed on the obesity index (OI), which is an aggregate genotypic value for the level of obesity of each individual pig. Several genes were detected (e.g., *NPC2*, *OR4D10*, and *CACNA1E*), and pathway analysis of all genomewide significant SNPs (404 SNPs) showed association with, e.g., insulin and immune system pathways. Second, 2500 SNPs were selected based on GWAS results (P -value < 0.05) and their connectivity to construct the WISH network. Pathway analysis of detected modules resulted in pathways related to, for example, metabolic processes and purinergic receptor activity. Lastly, a differentially wired network was constructed to detect potential obesity genes based on a differential connectivity. Here, genes such as *UBR1*, *PNPLA8*, and *CTNAP2* were detected, all of which are previously associated with obesity or obesity-related diseases.

Besides the genotyping in the UNIK pig resource population, a subset of the population was selected for RNA sequencing. The selection of pigs was obtained using selective expression profiling, based on the OI, selecting 12 extremely lean, 12 extremely obese, and 12 intermediate pigs. One of the small studies on this data set was the investigation of interaction between highly differentiating genes (lean vs. obese animals). They were visualized in Cytoscape (Shannon et al. 2003) and were further analyzed using the inbuild network analyzer (Fig. 2). We detected several genes that were so-called hub genes, and one clear example was *TNMD*. *TNMD* encodes a protein related to chondromodulin I, a cartilage-specific glycoprotein. Genetic variation in *TNMD* has been associated with central obesity and type 2 diabetes (Tolppanen et al. 2007).

The complete RNA-sequencing data were then used to construct coexpression networks using the weighted gene coexpression network analysis (WGCNA) approach, and regulator genes were detected using Lemon-Tree algorithms (Kogelman et al. 2014a). This resulted in the detection of several obesity-related pathways, but more interestingly in the detection of three potential causal genes linking obesity with osteoporosis: *CCR1*, *MSR1*, and *SPI1*. Continuing the systems genetics approaches using this UNIK pig resource population, one study focused on lncRNAs present among the genes in the previously detected WGCNA modules (Suravajhala et al. 2015). This study investigates those genes using the RNA-protein interaction predictor and detects network properties using Cytoscape

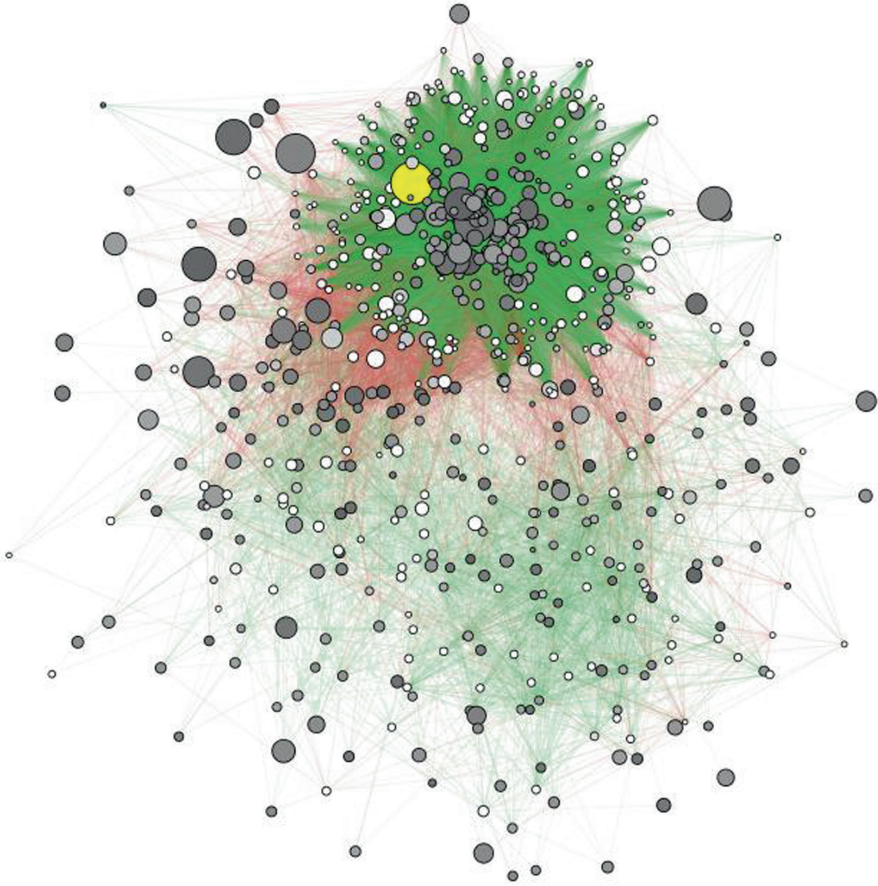


Fig. 2 Visualization of coexpression of genes selected based on their association with obesity, resulting from RNA-sequencing data of subcutaneous adipose tissue in the UNIK pig resource population. Edges represent Pearson's correlation coefficients between selected genes, colored on a *red–green* scale based on negative–positive correlation. The thicknesses of the edges are representing the strength of the correlations. Nodes represent selected genes, with size representing the fold change detected in differential expression analysis and darkness (*gray scale*) represents significance level of differential expression analysis. Only genes with a coexpression higher than 0.8 are visualized. *TNMD*, one of the selected hub genes, is colored *yellow*

(Shannon et al. 2003). They found that *cyp2c91* has strong interactions with several regulator genes, and they showed its importance in transcriptional regulation localized to cytoplasm. Furthermore, another study in progress within the UNIK population focuses on transcriptional regulation networks. This study extracted known transcription factors and used them as input of WGCNA to detect interaction patterns between transcription factors (Skinkyte-Juskiene et al. 2015).

Most recent research in the UNIK research population has been a study that integrates the genomic data with the transcriptomics data, using an eQTL mapping

approach. First, analysis of RNA-Seq data revealed 458 differentially expressed genes for the degree of obesity. Second, eQTL analysis revealed 987 cis-eQTLs and 73 trans-eQTLs. Data were further integrated by confining the eQTL mapping input to genomewide associated SNPs and differentially expressed genes. Furthermore, eQTL results were used for coexpression network construction. Many different obesity-related pathways and genes were identified, and several obesity candidate genes were proposed: *ENPP1*, *CTSL*, and *ABHD12B* (Kogelman et al. 2015).

8 Future Perspectives

Here we have described a selection of (ongoing) genetic research into obesity, using the pig as a biomedical model. The complexity of obesity, resulting from interactions between and within genes and environment, requires complex analyses of the different -omic levels to elucidate underlying genetic and biological mechanisms. Systems genetics approaches, a genetic branch of systems biology, offer those possibilities and are therefore an excellent choice for obesity research. Here we have focused on genomic and transcriptomic research, and besides the huge opportunities that still can be reached within those fields, there are also prospects for other -omics levels like epigenomics, metabolomics, and proteomics.

In human, epigenetic studies have shown its importance of detecting epigenetic changes underlying the development of obesity (van Dijk et al. 2015). A recently published study using a subset of pigs from the previously mentioned UNIK pig resource population focused on the epigenetic mechanisms in leukocytes (Jacobsen et al. 2016). They detected several genes that were differentially expressed (lean vs. obese pigs) in retroperitoneal adipose tissue and peripheral blood mononuclear cells. Several of those genes had a strong association with inflammatory pathways and fatty acid metabolism, as, for example, *SPPII*, *LEP*, and *INSIG1*. Also, metabolomics has shown its potential in human obesity research (Rauschert et al. 2014). A few metabolomic studies have been performed using pigs, with the aim to unravel metabolic mechanisms. He et al. (2012) found many differences in metabolite levels between lean and obese pigs, showing a distinct metabolism (e.g., difference in lipid oxidation and fermentation of gastrointestinal microbes) for obese pigs. Obesity research could highly benefit from the integration of metabolomics and genomic/transcriptomic data, to narrow down the unknown interactions between genetics and environmental factors. Proteomic studies have been used already more frequently in obesity research, using the pig as a model, and its potential is reviewed by Bendixen et al. (2010). It has been shown that several plasma proteins correlated well with obesity-related parameters (e.g., cholesterol and glucose) among pigs that were fed different diets (te Pas et al. 2013). Also, the protein expression of muscle in obese and lean pigs has been studied to gain knowledge about growth rate and meat quality in pigs, which can be used in human research (Li et al. 2013). They found metabolism-related proteins that were highly expressed in the obese pigs, but not in the lean pigs. Both proteins (COX5A and ATP5B) participate in oxidative phosphorylation. The protein that was highly expressed in the lean animals but not in obese pigs (ENO3) is involved in glycolysis.

Omic data generation (both microarray and sequence based) is getting easier and more affordable, and in combination with the ease of collecting samples from different cells/tissues in a porcine model, there is a huge potential to elucidate genetic and biological mechanisms of human obesity. Inherent in all these approaches are the dramatic increase in the use of omic-scale modeling and joint analyses of disparate multi-omic data sets using highly advanced bioinformatic and computational systems biology methods. This will become even more important and widespread in biomedical research in general and in complex diseases, such as obesity, in particular. As shown throughout this chapter, in the field of systems biology/systems genetics, there are many untouched areas in obesity research using a porcine model, which promises further growth and good prospective in the future.

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