HHS Public Access

Author manuscript

Annu Rev Anim Biosci. Author manuscript; available in PMC 2015 June 09.

Published in final edited form as:

Annu Rev Anim Biosci. 2013 January ; 1: 203–219. doi:10.1146/annurev-animal-031412-103715.

Genetically Engineered Pig Models for Human Diseases

Randall S. Prather[1], **Monique Lorson**[2], **Jason W. Ross**[3], **Jeffrey J. Whyte**[1], and **Eric Walters**[1]

[1] Division of Animal Science, National Swine Resource and Research Center

[2]Department of Veterinary Pathobiology, University of Missouri, Columbia, Missouri 65211

[3] Department of Animal Science, Iowa State University, Ames, Iowa 50011

Abstract

Although pigs are used widely as models of human disease, their utility as models has been enhanced by genetic engineering. Initially, transgenes were added randomly to the genome, but with the application of homologous recombination, zinc finger nucleases, and transcription activator-like effector nuclease (TALEN) technologies, now most any genetic change that can be envisioned can be completed. To date these genetic modifications have resulted in animals that have the potential to provide new insights into human diseases for which a good animal model did not exist previously. These new animal models should provide the preclinical data for treatments that are developed for diseases such as Alzheimer's disease, cystic fibrosis, retinitis pigmentosa, spinal muscular atrophy, diabetes, and organ failure. These new models will help to uncover aspects and treatments of these diseases that were otherwise unattainable. The focus of this review is to describe genetically engineered pigs that have resulted in models of human diseases.

Keywords

genetic engineering; cloning; swine

IMPORTANCE OF PIGS AS BIOMEDICAL MODELS

Treatment of humans as a result of disease or to repair injuries may appear straightforward. However, development of treatments or therapies requires a basic understanding of the biological condition. In addition, the recommended treatment or therapy may be technically difficult or complicated to implement. The disease or trauma often can be replicated in another species, in which case invasive description and intervention beyond what could be accomplished in humans can yield clues about the basic biology of the condition. These clues result in improved treatments or therapies that can be tested first on the model organism. Heavily used model systems include the mouse and rat. However, for some

PratherR@missouri.edu, LorsonM@missouri.edu, WhyteJ@missouri.edu, WaltersE@missouri.edu, JWRoss@iastate.edu

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

diseases and conditions, the mouse and rat are not appropriate. In fact, very few models completely replicate conditions in humans. A germane quote by George E. P. Box (1, p.74) asks us to, “Remember that all models are wrong; the practical question is how wrong do they have to be to not be useful.” Although the context for this quote regards building statistical models, the quote also extends to biological models. An example built upon later in this review is that of cystic fibrosis (CF). Mutations in a chloride ion channel, *CFTR* (CF transmembrane conductance regulator), result in CF. Mutation of this same gene in mice results in a defective chloride ion channel, and tissues from these mice have proven very useful for the study of ion channel function. Unfortunately, the mice don't develop the disease symptoms of CF. Thus, although the mouse model of CF is quite useful to study ion channels, it is not very useful to study CF as a disease.

Pigs also have advantages over other animal models in that societal concern presumably is lower for utilization of a food animal as a research model in comparison with companion animals. In many cases, wild-type pigs are being used as models already. Wild-type pigs are particularly useful for studying cardiovascular disease (2), atherosclerosis (3), cutaneous pharmacology (4), wound repair (5), cancer (6), diabetes (7), and ophthalmology (8). In many cases, they are the species of choice for translational medicine (9, 10). The National Institutes of Health considers pigs to be so important that it has established the National Swine Resource and Research Center at the University of Missouri (see **http://nsrrc.missouri.edu/**) to serve as a genetic resource for the biomedical community. Whereas other reviews have focused on the use of pigs in biomedical research in general (11, 12), the goal of this review is to describe genetically engineered pigs that have resulted in models of human diseases.

OVERVIEW OF GENETIC ENGINEERING IN PIGS

The first genetic modification of pigs was mediated by pronuclear injection (13). To accomplish this modification, a DNA construct was injected directly into the pronucleus of a pig zygote (**Table 1**). This technique permits the addition of a large transgene at a random location. In addition to genetic engineering by pronuclear injection, other techniques such as sperm-mediated transfection (14), oocyte transduction (15), and intracytoplasmic sperm injection (ICSI)–mediated transgenesis (16, 17) have been reported. Each of these techniques results in random integration. Although sperm- and ICSI-mediated transgenesis can use large constructs, transduction is limited by the physical constraints of the viral system. In contrast, transfection or transduction of somatic cells and selection, followed by somatic cell nuclear transfer (SCNT), permits selection of donor cells that have the desired integration prior to making the pig. The first transgenic SCNT-derived pigs were reported after viral transduction (18) or after homologous recombination (19). Genetic modification of the donor somatic cells for SCNT can be accomplished by most of the standard techniques that are used for genetic modification in other systems. Such techniques include homologous recombination (19), zinc finger nuclease–mediated modification (20, 21), transposases [Sleeping Beauty (22) and piggyBac (23)], transcription activator-like effector nucleases (TALENs) (24), adeno-associated viruses (25), replication-defective retroviruses (18), and lentiviruses (26). Most any method that can be used to genetically engineer a somatic cell can be used in combination with SCNT to create pigs with the desired

modification (**Figure 1**). For an exhaustive review of all the genetic modifications that have been completed in pigs, see Whyte & Prather (12).

SWINE GENOME SEQUENCING

One of the challenges to genetically engineering pigs is that it can be difficult to predict success. If you have an incomplete picture of the background genetics, gene knock-in/knockout is virtually impossible, especially in the case of a multigene family. Because the human and pig genome are relatively similar, intelligent predictions can be made. But without a base genome, they are just that, predictions. The lack of a sequenced genome is less important for adding a transgene but is still useful for designing the transgene to function as needed. Thus the sequenced genome (Ensembl Sscrofa 10.2, see **http://useast.ensembl.org/Sus_scrofa/Info/Index**) provides the background information needed to design targeting constructs so that they can not only effectively target a gene but also result in the desired transcripts, proteins, and subsequent phenotype (11).

MODELS THAT ARE MAKING A DIFFERENCE

Xenotransplantation

One of the pushes to create pigs with targeted genetic modifications came from the need to address the shortage of organs for transplantation to humans. The demand for organs far outstrips the supply; the number of people on the waiting list is over 110,000, and the number of transplants in 2011 was less than 29,000 (**http://www.unos.org/**). Twice the number on the waiting list could benefit from an organ transplant but are not ill enough to get on the list. One way to increase the number of available organs is to get them from another species of animal, such as the pig. Unfortunately there are cell-surface molecules in pigs to which humans and nonhuman primates have preexisting antibodies as well as other molecules that elicit an immune response. Those preexisting antibodies recognize a galactose α-1,3-galactose carbohydrate linkage and bind that epitope. Within minutes the complement proteins are recruited and the cells or organ are rejected. This is termed hyperacute rejection. The gene responsible for making the enzyme that catalyzes the formation of this carbohydrate structure is α-1,3-galactosyltransferase 1 (*GGTA1*). Although *GGTA1* is functional in pigs, in humans *GGTA1* is a pseudogene. Thus, more than 10 years ago considerable effort was put forth to create the technology to knock out *GGTA1* so that those preexisting antibodies would not result in hyperacute rejection. The only technology that had the potential at the time was homologous recombination in somatic cells followed by SCNT. The first addition of a transgene followed by SCNT (18) showed the potential to knock out a gene. Knockout of the first allele of *GGTA1* in pigs was reported in 2002 (19), and later homozygous animals were reported (27, 28). The good news was that knocking out *GGTA1* practically eliminated the hyperacute rejection. The bad news was that the other hurdles to the technology had become apparent. Those hurdles include post-hyperacute rejection (acute vascular rejection), cell-mediated rejection, nonvascular rejection (neurodegenerative disorders), and porcine endogenous retroviruses. Many different genetic modifications have been developed and proposed to deal with these hurdles. Because the focus of this review is on biomedical models, an exhaustive discussion is beyond our scope, and the reader is encouraged to consult other reviews on the topic (11, 12).

Cystic Fibrosis

An excellent example of the usefulness of genetically engineered pigs has been the development of pigs with a mutated *CFTR* gene. The CFTR protein is an ion channel that mediates hydration by regulating chloride ion transport. As stated in the introduction, CF is an autosomal recessive disorder. Mutations in *CFTR* in humans occur in approximately 5% of the population and are thus the most prevalent genetic mutations in North American adolescents (29). In addition, 70% of the individuals with CF have a deletion of the 508th amino acid (phenylalanine) of the CFTR protein (ΔF508). Approximately 15% of CF patients are born with meconium ileus (a blockage of the intestine), and some have a blocked pancreatic duct and focal biliary cirrhosis. They also develop a congealed gallbladder, a blocked bile duct, and a blockage of the vas deferens as well as lung disease. Mutating *CFTR* in the mouse so that it can no longer transport chloride ions does not result in the appearance of these classic CF symptoms. However, either deletion of *CFTR* or introduction of a ΔF508 in the pig results in 100% of the pigs having meconium ileus, destruction of the pancreas, liver lesions, a congealed gallbladder, blocked bile duct, blocked vas deferens, airway structural abnormalities, and lung disease (30–32). This model of CF additionally has led to the understanding of one of the most basic questions of CF: Which comes first in the development of lung disease, inflammation or infection? Because patients with CF present themselves to their physician and have both infection and inflammation, it has not been clear what should be treated; in other words, do people with mutations in *CFTR* have an underlying inflammation as a result of the mutation? Pigs with either a knockout of *CFTR* or the ΔF508 are born with sterile, noninflamed lungs and have difficulty in clearing bacterial invasion. This difficulty in clearing bacteria results in infection, and then inflammation follows that infection (33). Thus physicians now know that their treatments should focus on treating infection because if that clears up, the inflammation will also clear up. Observations of these mutated pigs also showed that their small stature is correlated with a low level of insulin-like growth factor 1 (IGF1) (34), and subsequent measurements on human CF patients confirmed that they too have low levels of IGF1 (34). Thus the pig has been demonstrated to be an excellent model of CF and likely will contribute to improved treatments and therapies in the near future.

Alzheimer's Disease

In 1906, German physician Alois Alzheimer described a progressive brain disorder that has come to be known as Alzheimer's disease (AD). It is characterized by loss of memory, confused thinking, and disorientation. In the United States alone, some 5 million people suffer from this form of dementia. Although the cause is not entirely known, a genetic link to the presenilin genes 1 and 2, as well as the amyloid precursor protein (*APP*) and tau protein, has been made (35, 36). In patients with AD, β-amyloid builds up between nerve cells in the brain and develops into plaques, and the microtubule-binding protein tau results in twisted fibers in neurons. To develop a model of AD in the pig, a transgene containing the so-called Swedish mutation (*APP695*sw), which lacks exons 7 and 8 (37), a β-globin sequence to induce splicing, and a PDGFβ promoter, was introduced into pigs (38). This resulted in a single random integration and expression of both message and protein in the brain. Unfortunately, the insertion is into the *GLIS3* gene (intron 5). Should this knock out

GLIS3, the results of homozygous *APP695*sw pigs will be confounded with this autosomal recessive birth defect (39). The AD research community is eagerly anticipating the disease phenotype of these pigs.

Diabetes

Diabetes is a group of metabolic disorders characterized by hyperglycemia. The two types of diabetes, type I and type II, both result in increased blood glucose but differ in the mechanism that causes the increase. Type I diabetes, which generally begins during adolescence (juvenile-onset diabetes), is a disorder in which the body attacks and destroys the insulin-producing cells, causing a decrease in the amount of insulin that can be produced to regulate blood glucose levels. In type II diabetes, the most common type, the cells in the body become resistant to insulin, and eventually the pancreas cannot produce enough insulin to overcome this resistance. Two key hormones, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), enhance insulin secretion in a glucose-dependent manner that is altered in diabetic patients.

The method of choice to treat patients with type I diabetes is to provide an exogenous source of insulin, generally by way of insulin injection. In an attempt to develop another route of administration, a pig has been developed that has a transgene containing a *GIP* promoter that drives expression of the human insulin gene. This results in human insulin production and secretion by the intestinal K cells (40). The next step in the project is to destroy the pig's pancreatic islet cells and determine if the pigs can regulate glucose levels based on the transgene as the only source of insulin. If so, this would be a preclinical model for transfecting the intestinal cells of patients with type I diabetes.

Transgenic pigs expressing a dominant-negative GIP receptor ($GIPR^{dn}$) in the pancreatic islets were generated (41) to resemble the characteristic features of human type II diabetes. As early as 11 weeks of age, these pigs exhibited a decreased oral glucose tolerance owing to delayed insulin release. In addition, at 11 weeks of age there was a 60% reduction of β-cell proliferation compared with controls (41). Furthermore, there was a reduction in β-cell mass in the $GIPR^{dn}$ pigs compared with controls in an age-dependent matter.

In addition to insulin-dependent diabetes, another type of diabetes, type III, is early-onset, noninsulin-dependent, and characterized by autosomal dominant inheritance (42, 43). Type III may be caused by a mutation in the *hepatocyte nuclear factor (HNF)-1α* gene encoding a transcriptional factor expressed in the liver, kidney, small intestine, spleen, and pancreas. Umeyama et al. (44) developed a diabetic pig model that expressed the dominant-negative mutant *HNF-1α.* Although they had a high mortality before weaning, many pigs lived long enough to be characterized with diabetes. This was confirmed with blood glucose levels greater than 200 mg/dl. In addition, histochemical analysis of the pancreas exhibited small and irregular islet cells, which thus resulted in poor insulin secretion. These models appear to recapitulate the features of human diabetes, thus paving the path to many translational experiments. Novel techniques, therapeutic strategies, and in vivo monitoring of pancreatic islet mass could be developed in conjunction with these large animal models.

Cardiovascular Disease

The surgical community has recognized swine to be an excellent anatomical and physiological model for the human cardiovascular system for decades (2, 45). The pig heart, coronary vasculature, and blood flow are very similar to those of the human, and overall, pigs are better suited to study cardiovascular disease than rodent and canine models (46). Atherosclerosis and myocardial infarction can be induced in swine by providing diets with nutrient compositions that are known to elevate the risk for cardiovascular disorders in humans (47). Some of the greatest potential advances in the study of cardiovascular disease lie in the development of genetically modified pigs to mimic human disorders or to enhance the study of disease etiology. The advent of SCNT to produce cloned swine and the sequencing of the porcine genome have enabled research studies in genetically identical swine that all harbor a gene variant associated with increased risk for atherosclerosis, such as mutant forms of *apoE4*, which are associated with severe type V hyperlipoproteinemia (48).

The first swine models produced with cardiovascular importance had integrated transgenes for desaturases that mammals lack but that are required for synthesis of Δ12 (n-6) and n-3 fatty acids. Transgenic pigs were developed to express Δ12 fatty acid desaturase (FAD2) from spinach (*Spinacia oleracea*) (49) to increase linoleic acid, and a separate pig model was produced to express a humanized *Caenorhabditis elegans* gene, *fat-1*, which encodes an n-3 fatty acid desaturase to increase the n-3/n-6 fatty acid ratio in meat (50). These pig models can be used to examine the cardiovascular effects of an altered n-3/n-6 fatty acid ratio in the swine compared with wild-type littermates. In the future, such genetically modified swine may provide food sources with cardiovascular-protective qualities.

Endothelial nitric oxide synthase (*eNOS*, or *NOS3*) in the inner lining of blood vessels generates nitric oxide (NO), an important signaling molecule for vasodilation and a regulator of vascular health (51). Transgenic swine that overexpress *eNOS* (52, 53) will increase our understanding of the role of NO in the complex regulation of vasodilation and may lead to therapies for diseases related to endothelial dysfunction. Hydrogen peroxide (H_2O_2) is closely involved in regulating vascular signaling by NO in the endothelium and is a critical molecule for proper cardiovascular regulation (54). The role of H_2O_2 in the development of vascular disorders and cardiac pathology associated with aging are not understood fully (55). To investigate the vascular role of H_2O_2, transgenic Yucatan minipigs were developed that overexpress human catalase in the endothelium (56). Catalase is the major enzyme responsible for catalyzing the decomposition of H_2O_2 to oxygen and water, so this transgenic pig model may provide further insight into the contribution of H_2O_2 to diseases such as atherosclerosis and preeclampsia. Recently, Yang et al. (57) combined zinc finger–nuclease technology with SCNT to produce knockout pigs with a disruptive mutation in peroxisome proliferator-activated receptor-γ (*PPAR-γ*). *PPAR-γ* is expressed in adipocytes, skeletal muscle, liver, and kidney, and PPAR-γ activation results in an increase in insulin sensitivity and glucose uptake as well as adiponectin and fatty acid uptake, in addition to anti-inflammatory effects (58). Examination of cardiovascular effects in *PPAR-γ* knockout pigs may lead to new strategies for therapeutic intervention. With all of the genetically modified pig cardiovascular models, real-time measurement of functional parameters like

blood flow, temperature, tissue oxygenation, perfusion, and diffusion can be conducted with existing instrumentation that is used on human patients. Such evaluations are difficult or impossible to administer in similar genetically modified rodent models.

Retinitis Pigmentosa

Retinitis pigmentosa (RP) refers to a large group of hereditary retinal diseases that impair affected individuals initially through night blindness, followed by loss of peripheral vision and, eventually, loss of central vision. Genetically, numerous mutations are known to result in RP in humans (see RetNet, **http://www.sph.uth.tmc.edu/RetNet/home.htm**), which makes it difficult to create animal models for the disease with the greatest potential for use in translational medicine. Despite the genetic heterogeneity of RP, a large proportion—approximately 25%—of autosomal dominant forms of RP are linked to mutations in the rhodopsin (RHO) gene.

Numerous animal models exist for the characterization of RP and the development of therapeutic interventions, including mice (59, 60), rats (61), dogs (62), and now two swine models (63, 64). Each animal model offers unique strengths and weaknesses with regard to the characterization of and development of intervention strategies for RP. The primary advantage of the swine models for RP is that they overcome the limitations of rodents because their eyes are of a more similar size to humans. Size is a critically important feature for development of interventions based on either cell transplantation or gene therapy, in which optimization of cell number and viral titer delivery, respectively, should be optimized in a large animal model prior to a clinical trial with humans. Additionally, having a cone-dominant central visual streak with a peripheral retina that is enriched with rods, the pig has a more similar retinal morphology compared with humans than rodents (65–68).

Of the two existing swine models of RP, both are transgenic and represent *RHO* mutations. The first, a P347L mutation in the swine *RHO* gene, was created in a domestic swine breed using pronuclear injection (64). The second swine model was created by stably integrating the human P23H *RHO* gene in the genome of somatic cells followed by SCNT to create an inbred miniature swine model of RP (63). The strategy used by Ross et al. (63) resulted in the production of six different founders that differed in transgene integration site and copy number. This variety ultimately resulted in a great degree of variation in the onset and progression of RP in these animals, an important characteristic in that disease onset and progression in humans is also highly variable. The miniature swine model is available to investigators through the National Swine Resource and Research Center (see **http://www.nsrrc.missouri.edu/**). Although both swine models are valuable research assets, the miniature swine model offers an additional advantage in that it is inbred. Because they have a defined major histocompatibility complex (MHC) haplotype, the cell transplantation can be conducted between animals while minimizing the probability of immunological rejection. The miniature swine model is also maintained more easily for long-term studies as a result of the reduced growth rate and smaller mature size in comparison with conventional swine breeds.

In addition to the two transgenic swine models of RP, a chemically induced RP model in swine has been developed (69). The authors administered a single iodoacetic acid bolus,

evaluated retinal morphology and function 12 weeks later, and demonstrated altered retinal morphology and function resembling the presentation of RP as a result of a genetic anomaly. This model is advantageous in that it offers rapid retinal degeneration and could be used for the development of intervention strategies that are based on cell transplantation (70); however, development and optimization of other strategies such as gene therapy, which specifically target and suppress the mutated *RHO* gene (71), will require a large animal model with a genetic basis for the disease.

Spinal Muscular Atrophy

Spinal muscular atrophy (SMA), an autosomal-recessive neurodegenerative disease, is the leading genetic cause of infantile death. SMA is characterized by loss of lower spinal motor neurons, skeletal muscle atrophy, paralysis, respiratory and gastrointestinal complications, scoliosis, and in many cases, a shortened life span. SMA presents in a broad clinical spectrum based upon the severity of symptoms and the ability to achieve physical milestones. There are three primary types (types I–III) of SMA (72, 73).

SMA is a result of a deletion or mutation of the *SMN1* (survival motor neuron) gene (74, 75). A nearly identical gene, *SMN2*, is present in one or more copies. Despite its sequence identity, *SMN2* is unable to prevent SMA disease development owing to an alternative splice event in which the majority of *SMN2*-derived transcripts lack exon 7 (Δ7). As a result, *SMN1* generates nearly 100% full-length SMN transcripts and full-length SMN protein while *SMN2* generates ~10% full-length transcripts (Figure 2). *SMN2* Δ7 transcripts produce a truncated SMN protein that is unstable and rapidly degraded (76). SMA therefore is a result of reduced SMN, not its complete absence, and the full-length SMN produced by *SMN2* serves to modify disease severity. Patients with one or two *SMN2* copies typically have the most severe forms of the disease, whereas increased copy number of *SMN2* is associated with decreased disease severity.

The most well-defined biochemical function of the SMN protein is in the assembly of the splicing-complex small nuclear ribonuclear proteins (77, 78). More recent investigations, however, have provided evidence for an additional SMN role in the stabilization and maturation of the neuromuscular junction and in neurotransmission (79–81). To date, it is unclear which cellular function is linked directly to SMA development because SMN-dependent splicing defects need further investigation, and a well-defined neuronal function has yet to be identified.

Deficiencies in SMN have been obtained in different model systems from worms to mice (82). Although each animal model has contributed to our understanding of SMN function, the various mouse models have been the most utilized. Humans are the only species with *SMN1* and *SMN2* genes; therefore, homozygous loss of *SMN* in all other animals results in embryonic lethality. To circumvent the lethal phenotype, many transgenic SMA models express the human *SMN2* transgene.

SMA therapeutic development has advanced rapidly, with several therapeutic strategies entering phase I clinical trials and the launch of the NeuroNext Biomarker Program (**http://www.neuronext.org**). Although SMA mouse models recapitulate many clinical features of

SMA, there remain disease and biological traits that restrict the utility of these mouse models in translational applications. To provide a model to evaluate dosing, delivery, distribution, sustained response, toxicity, and immune response in a clinically relevant manner, two pig models of SMA are being generated, the SMA Pig Splicing Model and the SMA Pig Disease Model.

The process of generating a SMA pig model was confounded by the requirement of the *SMN2* transgene and because SMN expression from *SMN2* needed to vary to represent the levels of SMA disease severity. As a result, the first pig *SMN* allele was deleted (Figure 3) and then the human *SMN2* transgene was added before the second pig allele could be targeted. Because SMN was expressed abundantly in pig fibroblasts, targeting pig *SMN* using homologous recombination and a promoter-trap strategy was possible; however, multiple attempts resulted in no targeting events. After utilizing multiple *SMN* targeting sequences and evaluating several primary cells without obtaining a gene-targeting event, single-stranded *SMN* DNA was generated to enhance recombination. For this *SMN* targeting sequence, single-stranded DNA successfully targeted the allele, and the first *SMN* knockout pigs were generated using SCNT (83). To generate $SMN^{+/\pm}$ pigs expressing human *SMN2*, $SMN^{+/-}$ primary cells were used for transfection of a 35.5-kb fragment carrying the SMN2 gene and its promoter sequence. Multiple positive clones expressing SMN from *SMN2* were obtained and used to generate $SMN^{+/-}$; h*SMN2* pigs by SCNT. These pigs serve as the genetic context for the SMA Pig Splicing Model and will be used to generate the SMA Pig Disease Model by breeding.

The SMA Pig Splicing Model ($SMN^{+/-}$;hSMN2) serves as an initial model to evaluate delivery and test efficacy of therapeutics that modulate the splicing of *SMN2*. The benefit of this model is that the animals are phenotypically wild type; therefore, delivery and therapeutic modulation of *SMN2* splicing patterns as a result of therapeutic delivery can be measured easily without battling disease severity. Several therapeutics that have demonstrated efficacy in SMA mouse models will be tested in this pig model. The SMA Pig Disease Model ($SMN^{-/-}$;h*SMN2*) will also be based upon the human *SMN2* gene but will lack pig SMN and will therefore serve as a valuable model to evaluate delivery and efficacy of any SMA therapeutic. The goal is to obtain SMA disease pigs that will represent each severity group of SMA (types I–III). The first set of SMA disease pigs is currently in development.

Huntington's Disease

Huntington's disease (HD) results from expansion of a trinucleotide (CAG) repeat in a gene called Huntingtin (*HTT*) (84). The number of repeats in normal individuals ranges from 11 to 34, and when the number of repeats exceeds 35, mild symptoms appear, whereas when there are over 40 repeats, all individuals have more severe symptoms (85). Because CAG repeats longer than 28 tend not to replicate accurately during DNA synthesis, new expansions can be generated spontaneously. Thus over generations the size of the repeat can increase, and size is more unstable during spermatogenesis versus oogenesis. Because CAG codes for glutamine (Q), expansion of the CAG trinucleotide repeat results in an aberrant Huntingtin protein that has an extended polyQ tract. These polyQ tracts, which result in

misfolded protein, are thought to alter mitochondrial function or intracellular signaling and induce apoptosis (86–88). HD is characterized by a progressive breakdown of brain neurons, and symptoms include disorders of movement, decreased cognitive abilities, and psychiatric disorders (depression, mania, bipolar disorder, obsessive-compulsive behaviors) (89). In comparison, dementia in HD is subcortical, whereas dementia in AD is cortical.

Mouse and monkey models currently are available for HD. Transgenic mouse models for HD have been invaluable for the pathogenesis of the disorder but lack one of the hallmark phenotypes of HD: apoptosis of the neurons, i.e., neurodegeneration. This is believed to be a result of differences in rodent neural anatomy and gene function compared with humans (90). Nonhuman primates have brains similar to humans in terms of anatomy and function, with an additional advantage over the rodent of the ability to perform cognitive, social, and motor tests (90).

At least two lines of transgenic swine have been produced for the study of HD. The first was produced by pronuclear injection and contained a 75-polyQ tract but had no phenotype (91). A second group introduced a 105-polyQ tract into somatic cells and then created the pigs by SCNT (92). Some of these pigs had symptoms resembling HD, e.g., chorea-like movement and apoptotic neurons in the brain. When the authors tried to increase the length of the polyQ tract to 160, the surrogates were unable to produce piglets. They concluded that in the pig, the polyQ tract needs to be over 75 and less than 160 to obtain a phenotype. Unlike the mouse model expressing the same transgene, these pigs presented apoptotic neurons (exhibiting DNA fragmentation) in their brains. The pigs additionally expressed more cells with activated capase-3 activity that had neuronal specificity as peripheral tissues had no activated capase-3 activity. The neuronal-specific capase-3 activity also was located in the striatum and not the cortex region of the brain. This is another example in which the pig model may be the most appropriate model for HD; in contrast to the mouse, the pig exhibits DNA fragmentation and apoptotic neurons in the brain that are typical of those observed in humans.

Cancer

Cancer is a generic name for a large group of more than 100 diseases in which a cell in the body begins to grow uncontrollably. Cells usually become abnormal as a result of DNA damage, which allows them to continue to grow and divide as well as invade the surrounding tissue. These characteristics are the definition of a cancer cell, and specific cancers are named for the location from which they begin. It is estimated that approximately 850,000 men and ~800,000 women in the United States will have some form of cancer in 2012 (93). Approximately 29% of the 850,000 of the men will have prostate cancer, and 29% of the 800,000 women will have breast cancer; lung cancer is second most common for both men and women (93). Genomic instability, activation of oncogenes, and inactivation of tumor-suppressor genes can vary with the different types of cancer. Although there is at least one report of a transgenic pig for use as a cancer model [mammary tumor (94)] its usefulness has not been demonstrated. Induction of genetically defined tumors in a tissue-specific manner can be accomplished without genetically engineering the pig (95). Nevertheless, the National Swine Resource and Research Center is collaborating with a

couple of groups to develop cancer models including: (*a*) a generic cancer model (expression of mutated KRAS and p53) that can be induced tissue specifically and (*b*) a breast cancer model (microRNA knockout). These models have the potential to dramatically impact cancer biology by providing tools for the development of novel therapies, such as targeted epigenetic reprogramming with artificial transcription factors (96).

FUTURE AND CONCLUSIONS

The above review highlights many of the genetic modifications that have been reported to create models of human disease in pigs (Figure 4). Because creating and maintaining genetically modified pigs is very expensive, in most cases they are developed only as a result of deficiencies in other models. Some of those described are in the early stages of characterization, and it is not yet known how useful they will be for modeling specific diseases. Other models that are not discussed above, and which are in the very early stages of characterization, include muscular dystrophy (97), psoriasis-like phenotype (98), and osteoporosis (99). As the pig is shown to be a suitable model for diseases for which a good model does not exist currently, additional genetic modifications will continue to be made. Technological advances in the ability to precisely modify the genome and to create animals via in vitro techniques, such as SCNT, will contribute to more efficient production of these valuable animals. In addition, the more that is known about the genetic basis for the disease in humans, and the increased knowledge of the pig genome, will enable a more directed approach to the creation of these animals.

ACKNOWLEDGMENTS

Authors received funding from National Institutes of Health R01 RR013438, U42 RR018877/OD011140, R21 NSO595101, R21 RR039232, R21 EY020647, FightSMA, and the FightSMA/Gwendolyn Strong Foundation.

LITERATURE CITED

1. Box, GEP.; Draper, NR. Empirical Model Building. John Wiley & Sons; New York: 1987.
2. Turk JR, Laughlin MH. Physical activity and atherosclerosis: Which animal model? Can. J. Appl. Physiol. 2004; 29(5):657–83. [PubMed: 15536667]
3. Ishii A, Viñuela F, Murayama Y, Yuki I, Nien YL, et al. Swine model of carotid artery atherosclerosis: experimental induction by surgical partial ligation and dietary hypercholesterolemia. Am. J. Neuroradiol. 2006; 27(9):1893–99. [PubMed: 17032861]
4. Herkenne C, Naik A, Kalia YN, Hadgraft J, Guy RH. Pig ear skin *ex vivo* as a model for *in vivo* dermatopharmacokinetic studies in man. Pharm. Res. 2006; 23(8):1850–56. [PubMed: 16841197]
5. Graham JS, Reid FM, Smith JR, Stotts RR, Tucker FS, et al. A cutaneous full-thickness liquid sulfur mustard burn model in weanling swine: clinical pathology and urinary excretion of thiodiglycol. J. Appl. Toxicol. 2000; 20:S161–72. [PubMed: 11428630]
6. Du ZQ, Vincent-Naulleau S, Gilbert H, Vignoles F, Créchet F, et al. Detection of novel quantitative trait loci for cutaneous melanoma by genome-wide scan in the MeLiM swine model. Int. J. Cancer. 2007; 120(2):303–20. [PubMed: 17066441]
7. Dyson MC, Alloosh M, Vuchetich JP, Mokelke EA, Sturek M. Components of metabolic syndrome and coronary artery disease in female Ossabaw swine fed excess atherogenic diet. Comp. Med. 2006; 56(1):35–45. [PubMed: 16521858]
8. Noel JM, Fernandez de Castro JP, Demarco PJ Jr, Franco LM, Wang W, et al. Iodacetic acid, but not sodium iodate, creates an inducible swine model of photoreceptor damage. Exp. Eye Res. 2012; 97(1):137–47. [PubMed: 22251455]

9. Peters LL, Robledo RF, Bult CJ, Churchill GA, Paigen BJ, Svenson KL. The mouse as a model for human biology: a resource guide for complex trait analysis. Nat. Rev. Genet. 2007; 8(1):58–69. [PubMed: 17173058]
10. Trancikova A, Ramonet D, Moore DJ. Genetic mouse models of neurodegenerative diseases. Prog. Mol. Biol. Transl. Sci. 2011; 100:419–82. [PubMed: 21377633]
11. Walters EM, Wolf E, Whyte JJ, Mao J, Renner S, et al. Completion of the swine genome will simplify the production of swine as a large animal biomedical model. BMC Med. Genom. 2012; 5:55.
12. Whyte JJ, Prather RS. Genetic modifications of pigs for medicine and agriculture. Mol. Reprod. Dev. 2011; 78(10–11):879–91. [PubMed: 21671302]
13. Hammer RE, Pursel VG, Rexroad CE Jr, Wall RJ, Bolt DJ, et al. Production of transgenic rabbits, sheep and pigs by microinjection. Nature. 1985; 315(6021):680–83. [PubMed: 3892305]
14. Lavitrano M, Forni M, Varzi V, Pucci L, Bacci ML, et al. Sperm-mediated gene transfer: production of pigs transgenic for a human regulator of complement activation. Transplant. Proc. 1997; 29(8):3508–9. [PubMed: 9414813]
15. Cabot RA, Kühholzer B, Chan AW, Lai L, Park KW, et al. Transgenic pigs produced using in vitro matured oocytes infected with a retroviral vector. Anim. Biotechnol. 2001; 12(2):205–14. [PubMed: 11808636]
16. Lai L, Sun Q, Wu G, Murphy CN, Kühholzer B, et al. Development of porcine embryos and offspring after intracytoplasmic sperm injection with liposome transfected or non-transfected sperm into in vitro matured oocytes. Zygote. 2001; 9(4):339–46. [PubMed: 11771901]
17. Pereyra-Bonnet F, Fernández-Martín R, Olivera R, Jarazo J, Vichera G, et al. A unique method to produce transgenic embryos in ovine, porcine, feline, bovine and equine species. Reprod. Fertil. Dev. 2008; 20(7):741–49. [PubMed: 18842176]
18. Park KW, Cheong HT, Lai L, Im GS, Kühholzer B, et al. Production of nuclear transfer-derived swine that express the enhanced green fluorescent protein. Anim. Biotechnol. 2001; 12(2):173–81. [PubMed: 11808633]
19. Lai L, Kolber-Simonds D, Park KW, Cheong HT, Greenstein JL, et al. Production of α-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. Science. 2002; 295(5557):1089–92. [PubMed: 11778012]
20. Whyte JJ, Zhao J, Wells KD, Samuel MS, Whitworth KM, et al. Gene targeting with zinc finger nucleases to produce cloned eGFP knockout pigs. Mol. Reprod. Dev. 2011; 78(1):2. [PubMed: 21268178]
21. Whyte JJ, Prather RS. Cell Biology Symposium: Zinc finger nucleases to create custom-designed modifications in the swine (*Sus scrofa*) genome. J. Anim. Sci. 2012; 90(4):1111–17. [PubMed: 22038991]
22. Garrels W, Holler S, Struckmann C, Taylor U, Ehling C, et al. Analysis of fluorophore-expressing spermatozoa from transgenic boars produced by sleeping beauty transposition. Reprod. Fertil. Dev. 2011; 23:260.
23. Kim SJ, Koo OJ, Park SJ, Moon JH, Kwon DK, et al. Inducible fluorescent protein (RFP) expression in porcine fibroblasts and transgenic cloned embryos using piggyback transposition. Reprod. Fertil. Dev. 2011; 23:262.
24. Miller JC, Tan S, Qiao G, Barlow KA, Wang J, et al. A TALE nuclease architecture for efficient genome editing. Nat. Biotechnol. 2011; 29(2):143–48. [PubMed: 21179091]
25. Rogers CS, Hao Y, Rokhlina T, Samuel M, Stoltz DA, et al. Production of *CFTR*-null and *CFTR*-Δ*F508* heterozygous pigs by adeno-associated virus-mediated gene targeting and somatic cell nuclear transfer. J. Clin. Investig. 2008; 118(4):1571–77. [PubMed: 18324337]
26. Hofmann A, Kessler B, Ewerling S, Weppert M, Vogg B, et al. Efficient transgenesis in farm animals by lentiviral vectors. EMBO Rep. 2003; 4(11):1054–60. [PubMed: 14566324]
27. Kolber-Simonds D, Lai L, Watt SR, Denaro M, Arn S, et al. Production of α-1,3-galactosyltransferase null pigs by means of nuclear transfer with fibroblasts bearing loss of heterozygosity mutations. Proc. Natl. Acad. Sci. USA. 2004; 101(19):7335–40. [PubMed: 15123792]

28. Phelps CJ, Koike C, Vaught TD, Boone J, Wells KD, et al. Production of α1,3-galactosyltransferase-deficient pigs. Science. 2003; 299(5605):411–14. [PubMed: 12493821]
29. Welsh MJ, Rogers CS, Stoltz DA, Meyerholz DK, Prather RS. Development of a porcine model of cystic fibrosis. Trans. Am. Clin. Climatol. Assoc. 2009; 120:149–62. [PubMed: 19768173]
30. Meyerholz DK, Stoltz DA, Pezzulo AA, Welsh MJ. Pathology of gastrointestinal organs in a porcine model of cystic fibrosis. Am. J. Pathol. 2010; 176(3):1377–89. [PubMed: 20110417]
31. Rogers CS, Stoltz DA, Meyerholz DK, Ostedgaard LS, Rokhlina T, et al. Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. Science. 2008; 321(5897):1837–41. [PubMed: 18818360]
32. Meyerholz DK, Stoltz DA, Namati E, Ramachandran S, Pezzulo AA, et al. Loss of cystic fibrosis transmembrane conductance regulator function produces abnormalities in tracheal development in neonatal pigs and young children. Am. J. Respir. Crit. Care Med. 2010; 182(10):1251–61. [PubMed: 20622026]
33. Stoltz DA, Meyerholz DK, Pezzulo AA, Ramachandran S, Rogan MP, et al. Cystic fibrosis pigs develop lung disease and exhibit defective bacterial eradication at birth. Sci. Transl. Med. 2010; 2(29):29ra31.
34. Rogan MP, Reznikov LR, Pezzulo AA, Gansemer ND, Samuel M, et al. Pigs and humans with cystic fibrosis have reduced insulin-like growth factor 1 (IGF1) levels at birth. Proc. Natl. Acad. Sci. USA. 2010; 107(47):20571–75. [PubMed: 21059918]
35. Scheuner D, Eckman C, Jensen M, Song X, Citron M, et al. Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and *APP* mutations linked to familial Alzheimer's disease. Nat. Med. 1996; 2(8):864–70. [PubMed: 8705854]
36. McGurk L, Bonini NM. Yeast informs Alzheimer's disease. Science. 2011; 334(6060):1212–13. [PubMed: 22144604]
37. Neve RL, Finch EA, Dawes LR. Expression of the Alzheimer amyloid precursor gene transcripts in the human brain. Neuron. 1988; 1(8):669–77. [PubMed: 2908447]
38. Kragh PM, Nielsen AL, Li J, Du Y, Lin L, et al. Hemizygous minipigs produced by random gene insertion and handmade cloning express the Alzheimer's disease-causing dominant mutation APPsw. Transgenic Res. 2009; 18(4):545–58. [PubMed: 19184503]
39. Senée V, Chelala C, Duchatelet S, Feng D, Blanc H, et al. Mutations in GLIS3 are responsible for a rare syndrome with neonatal diabetes mellitus and congenital hypothyroidism. Nat. Genet. 2006; 38(6):682–87. [PubMed: 16715098]
40. Whyte, JJ.; Isom, SC.; Samuel, M.; Asadi, A.; Kieffer, TJ.; Prather, RS. Human Insulin Expression in Intestinal K-Cells of Transgenic Swine.. Presented at Swine Biomed. Res. Conf; Chicago, IL. July 17-19; 2011.
41. Renner S, Fehlings C, Herbach N, Hofmann A, von Waldthausen DC, et al. Glucose intolerance and reduced proliferation of pancreatic β-cells in transgenic pigs with impaired glucose-dependent insulinotropic polypeptide function. Diabetes. 2010; 59(5):1228–38. [PubMed: 20185813]
42. Yamagata K. Regulation of pancreatic β-cell function by the HNF transcription network: lessons from maturity-onset diabetes of the young (MODY). Endocr. J. 2003; 50(5):491–99. [PubMed: 14614204]
43. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, et al. Mutations in the hepatocyte nuclear factor-1α gene in maturity-onset diabetes of the young (MODY3). Nature. 1996; 384(6608):455–58. [PubMed: 8945470]
44. Umeyama K, Watanabe M, Saito H, Kurome M, Tohi S, et al. Dominant-negative mutant hepatocyte nuclear factor 1α induces diabetes in transgenic-cloned pigs. Transgenic Res. 2009; 18(5):697–706. [PubMed: 19357985]
45. Swindle, MM. Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques. CRC; Boca Raton, FL: 2007.
46. Vilahur G, Padro T, Badimon L. Atherosclerosis and thrombosis: insights from large animal models. J. Biomed. Biotechnol. 2011; 2011:907575. [PubMed: 21274431]
47. Young LH. Diet-induced obesity obstructs insulin signaling in the heart. Am. J. Physiol. Heart Circ. Physiol. 2010; 298(2):H306–7. [PubMed: 19940075]

48. Jensen TW, Mazur MJ, Pettigew JE, Perez-Mendoza VG, Zachary J, Schook LB. A cloned pig model for examining atherosclerosis induced by high fat, high cholesterol diets. Anim. Biotechnol. 2010; 21(3):179–87. [PubMed: 20665290]
49. Saeki K, Matsumoto K, Kinoshita M, Suzuki I, Tasaka Y, et al. Functional expression of a Δ12 fatty acid desaturase gene from spinach in transgenic pigs. Proc. Natl. Acad. Sci. USA. 2004; 101(17):6361–66. [PubMed: 15067141]
50. Lai L, Kang JX, Li R, Wang J, Witt WT, et al. Generation of cloned transgenic pigs rich in omega-3 fatty acids. Nat. Biotechnol. 2006; 24(4):435–36. [PubMed: 16565727]
51. Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, et al. Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. Nature. 1999; 399(6736):597–601. [PubMed: 10376602]
52. Hao YH, Yong HY, Murphy CN, Wax D, Samuel M, et al. Production of endothelial nitric oxide synthase (eNOS) over-expressing piglets. Transgenic Res. 2006; 15(6):739–50. [PubMed: 17080303]
53. Whyte JJ, Laughlin MH. Placentation in the pig visualized by eGFP fluorescence in eNOS over-expressing cloned transgenic swine. Mol. Reprod. Dev. 2010; 77(7):565. [PubMed: 20578058]
54. Drouin A, Thorin E. Flow-induced dilation is mediated by Akt-dependent activation of endothelial nitric oxide synthase-derived hydrogen peroxide in mouse cerebral arteries. Stroke. 2009; 40(5): 1827–33. [PubMed: 19286591]
55. Collins AR, Lyon CJ, Xia X, Liu JZ, Tangirala RK, et al. Age-accelerated atherosclerosis correlates with failure to upregulate antioxidant genes. Circ. Res. 2009; 104(6):e42–54. [PubMed: 19265038]
56. Whyte JJ, Samuel M, Mahan E, Padilla J, Simmons GH, et al. Vascular endothelium-specific overexpression of human catalase in cloned pigs. Transgenic Res. 2011; 20(5):989–1001. [PubMed: 21170678]
57. Yang D, Yang H, Li W, Zhao B, Ouyang Z, et al. Generation of PPARγ mono-allelic knockout pigs via zinc-finger nucleases and nuclear transfer cloning. Cell Res. 2011; 21(6):979–82. [PubMed: 21502977]
58. Nicholls SJ, Uno K. Peroxisome proliferator-activated receptor (PPAR α/γ) agonists as a potential target to reduce cardiovascular risk in diabetes. Diabetes Vasc. Dis. Res. 2012; 9(2):89–94.
59. Olsson JE, Gordon JW, Pawlyk BS, Roof D, Hayes A, et al. Transgenic mice with a rhodopsin mutation (Pro23His): a mouse model of autosomal dominant retinitis pigmentosa. Neuron. 1992; 9(5):815–30. [PubMed: 1418997]
60. Mao H, James T Jr, Schwein A, Shabashvili AE, Hauswirth WW, et al. AAV delivery of wild-type rhodopsin preserves retinal function in a mouse model of autosomal dominant retinitis pigmentosa. Hum. Gene Ther. 2011; 22(5):567–75. [PubMed: 21126223]
61. Ji Y, Zhu CL, Grzywacz NM, Lee EJ. Rearrangement of the cone mosaic in the retina of the rat model of retinitis pigmentosa. J. Comp. Neurol. 2012; 520(4):874–88. [PubMed: 22102145]
62. Kijas JW, Cideciyan AV, Aleman TS, Pianta MJ, Pearce-Kelling SE, et al. Naturally occurring rhodopsin mutation in the dog causes retinal dysfunction and degeneration mimicking human dominant retinitis pigmentosa. Proc. Natl. Acad. Sci. USA. 2002; 99(9):6328–33. [PubMed: 11972042]
63. Ross JW, Fernandez de Castro JP, Zhao J, Samuel M, Walters E, et al. Generation of an inbred miniature pig model of retinitis pigmentosa. Investig. Ophthalmol. Vis. Sci. 2012; 53(1):501–7. [PubMed: 22247487]
64. Petters RM, Alexander CA, Wells KD, Collins EB, Sommer JR, et al. Genetically engineered large animal model for studying cone photoreceptor survival and degeneration in retinitis pigmentosa. Nat. Biotechnol. 1997; 15(10):965–70. [PubMed: 9335046]
65. Szél A, Röhlich P, Caffé AR, Juliusson B, Aguirre G, Van Veen T. Unique topographic separation of two spectral classes of cones in the mouse retina. J. Comp. Neurol. 1992; 325(3):327–42. [PubMed: 1447405]
66. Roof DJ, Adamian M, Hayes A. Rhodopsin accumulation at abnormal sites in retinas of mice with a human P23H rhodopsin transgene. Investig. Ophthalmol. Vis. Sci. 1994; 35(12):4049–62. [PubMed: 7960587]

67. Beauchemin ML, Babel J. Proceedings: A preliminary study on the ultrastructure of the pig retina. Exp. Eye Res. 1973; 17(4):400. [PubMed: 4765291]
68. Braekevelt CR. Fine structure of the retinal rods and cones in the domestic pig. Graefes Arch. Clin. Exp. Ophthalmol. 1983; 220(6):273–78. [PubMed: 6629020]
69. Wang W, Fernandez de Castro J, Vukmanic E, Zhou L, Emery D, et al. Selective rod degeneration and partial cone inactivation characterize an iodoacetic acid model of Swine retinal degeneration. Investig. Ophthalmol. Vis. Sci. 2011; 52(11):7917–23. [PubMed: 21896868]
70. Tucker BA, Park IH, Qi SD, Klassen HJ, Jiang C, et al. Transplantation of adult mouse iPS cell-derived photoreceptor precursors restores retinal structure and function in degenerative mice. PLoS ONE. 2011; 6(4):e18992. [PubMed: 21559507]
71. Millington-Ward S, Chadderton N, O'Reilly M, Palfi A, Goldmann T, et al. Suppression and replacement gene therapy for autosomal dominant disease in a murine model of dominant retinitis pigmentosa. Mol. Ther. 2011; 19(4):642–49. [PubMed: 21224835]
72. Wee CD, Kong L, Sumner CJ. The genetics of spinal muscular atrophies. Curr. Opin. Neurol. 2010; 23(5):450–58. [PubMed: 20733483]
73. Prior TW. Perspectives and diagnostic considerations in spinal muscular atrophy. Genet. Med. 2010; 12(3):145–52. [PubMed: 20057317]
74. Wirth B. An update of the mutation spectrum of the survival motor neuron gene (SMN1) in autosomal recessive spinal muscular atrophy (SMA). Hum. Mutat. 2000; 15(3):228–37. [PubMed: 10679938]
75. Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P, et al. Identification and characterization of a spinal muscular atrophy-determining gene. Cell. 1995; 80(1):155–65. [PubMed: 7813012]
76. Lorson CL, Hahnen E, Androphy EJ, Wirth B. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. Proc. Natl. Acad. Sci. USA. 1999; 96(11): 6307–11. [PubMed: 10339583]
77. Kolb SJ, Battle DJ, Dreyfuss G. Molecular functions of the SMN complex. J. Child Neurol. 2007; 22(8):990–94. [PubMed: 17761654]
78. Paushkin S, Gubitz AK, Massenet S, Dreyfuss G. The SMN complex, an assemblyosome of ribonucleoproteins. Curr. Opin. Cell Biol. 2002; 14(3):305–12. [PubMed: 12067652]
79. Burghes AH, Beattie CE. Spinal muscular atrophy: Why do low levels of survival motor neuron protein make motor neurons sick? Nat. Rev. Neurosci. 2009; 10(8):597–609. [PubMed: 19584893]
80. Bowerman M, Murray LM, Beauvais A, Pinheiro B, Kothary R. A critical smn threshold in mice dictates onset of an intermediate spinal muscular atrophy phenotype associated with a distinct neuromuscular junction pathology. Neuromuscul. Disord. 2012; 22(3):263–76. [PubMed: 22071333]
81. Kong L, Wang X, Choe DW, Polley M, Burnett BG, et al. Impaired synaptic vesicle release and immaturity of neuromuscular junctions in spinal muscular atrophy mice. J. Neurosci. 2009; 29(3): 842–51. [PubMed: 19158308]
82. Park GH, Kariya S, Monani UR. Spinal muscular atrophy: new and emerging insights from model mice. Curr. Neurol. Neurosci. Rep. 2010; 10(2):108–17. [PubMed: 20425235]
83. Lorson MA, Spate LD, Samuel MS, Murphy CN, Lorson CL, et al. Disruption of the Survival Motor Neuron (*SMN*) gene in pigs using ssDNA. Transgenic Res. 2011; 20(6):1293–304. [PubMed: 21350916]
84. Gusella JF, Wexler NS, Conneally PM, Naylor SL, Anderson MA, et al. A polymorphic DNA marker genetically linked to Huntington's disease. Nature. 1983; 306(5940):234–38. [PubMed: 6316146]
85. Reiner A, Dragatsis I, Dietrich P. Genetics and neuropathology of Huntington's disease. Int. Rev. Neurobiol. 2011; 98:325–72. [PubMed: 21907094]
86. Yuan J, Yankner BA. Apoptosis in the nervous system. Nature. 2000; 407(6805):802–9. [PubMed: 11048732]
87. Mattson MP. Apoptosis in neurodegenerative disorders. Nat. Rev. Mol. Cell Biol. 2000; 1(2):120–29. [PubMed: 11253364]
88. Friedlander RM. Apoptosis and caspases in neurodegenerative diseases. N. Engl. J. Med. 2003; 348(14):1365–75. [PubMed: 12672865]

89. Walker FO. Huntington's disease. Lancet. 2007; 369(9557):218–28. [PubMed: 17240289]
90. Yang SH, Chan AW. Transgenic animal models of Huntington's disease. Curr. Top. Behav. Neurosci. 2011; 7:61–85. [PubMed: 21225414]
91. Uchida M, Shimatsu Y, Onoe K, Matsuyama N, Niki R, et al. Production of transgenic miniature pigs by pronuclear microinjection. Transgenic Res. 2001; 10(6):577–82. [PubMed: 11817545]
92. Yang D, Wang CE, Zhao B, Li W, Ouyang Z, et al. Expression of Huntington's disease protein results in apoptotic neurons in the brains of cloned transgenic pigs. Hum. Mol. Genet. 2010; 19(20):3983–94. [PubMed: 20660116]
93. Am. Cancer Soc.. Cancer Facts and Figures 2012. Am. Cancer Soc.; Atlanta, GA: 2012. http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-031941.pdf
94. Yamakawa H, Nagai T, Harasawa R, Yamagami T, Takahashi J, et al. Production of transgenic pig carrying MMTV/v-Ha-ras. J. Reprod. Dev. 1999; 45:111–18.
95. Adam SJ, Counter CM. A method to generate genetically defined tumors in pigs. Methods Enzymol. 2008; 439:39–51. [PubMed: 18374155]
96. Rivenbark AG, Stolzenburg S, Beltran AS, Yuan X, Rots M, et al. Epigenetic reprogramming of cancer cells via targeted DNA methylation. Epigenetics. 2012; 7(4):350–60. [PubMed: 22419067]
97. Klymiuk N, Thirion C, Burkhardt K, Wuensch A, Krause S, et al. Tailored pig model of Duchenne muscular dystrophy. Reprod. Fertil. Dev. 2012; 24:231.
98. McCalla-Martin AC, Chen X, Linder KE, Estrada JL, Piedrahita JA. Varying phenotypes in swine versus murine transgenic models constitutively expressing the same human Sonic hedgehog transcriptional activator, *K5-HGL12ΔN*. Transgenic Res. 2009; 19:869–87. [PubMed: 20099029]
99. Klymiuk N, Böcker W, Schönitzer V, Bähr A, Radic T, et al. First inducible transgene expression in porcine large animal models. FASEB J. 2012; 26(3):1086–99. [PubMed: 22138035]

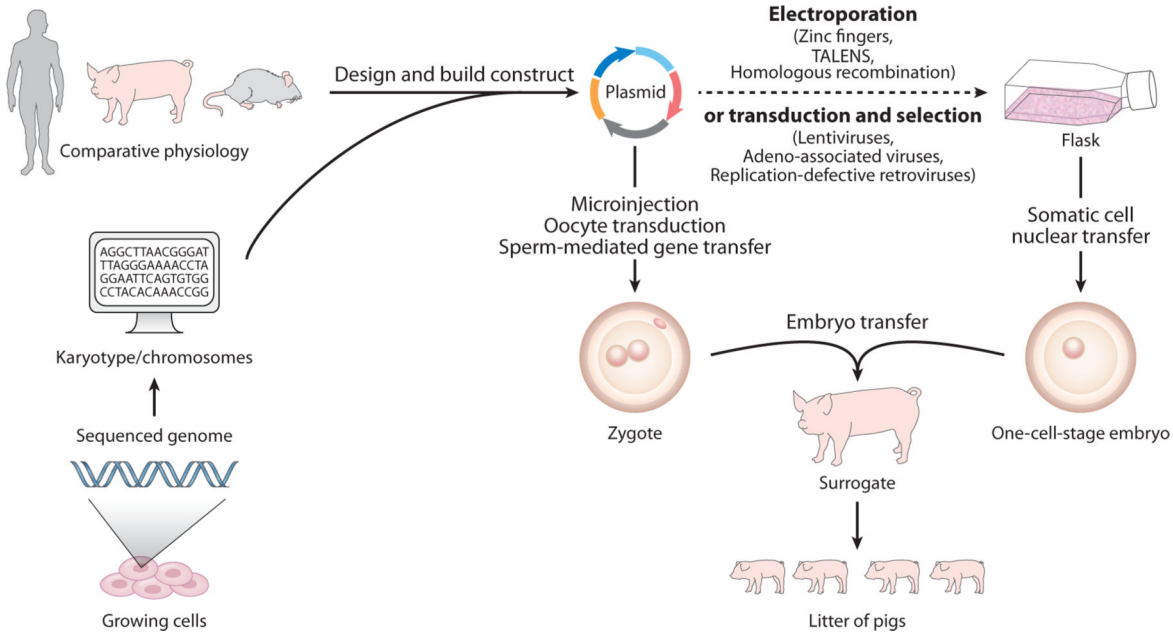

Figure 1.

Overview of genetic engineering in the pig. DNA is isolated from growing cells. By using a comparative approach between humans and other species, constructs that are predicted to create a pig with the desired phenotype are designed and built. Once the construct is engineered, it is used to transduce oocytes, to microinject into pronuclei, or to mix with sperm for sperm-mediated gene transfer, or it is introduced into somatic cells by electroporation or transduction. After the desired stable integration into the somatic cells is determined, somatic cell nuclear transfer is used to create embryos. Embryos with genetically engineered genomes are transferred to a surrogate mother, which then carries the pregnancy to term and delivers one to ten piglets with the desired genetic modification(s).

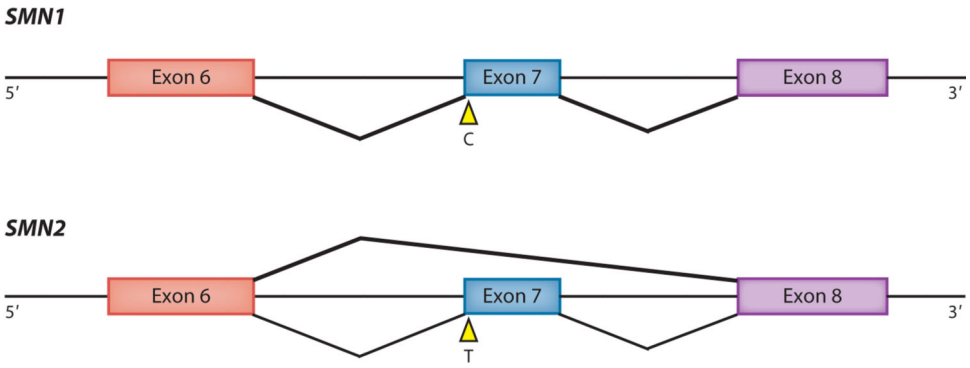

Figure 2.

Human *SMN1* generates almost exclusively full-length *SMN* transcripts. Human *SMN2*, owing to a C-to-T transition in exon 7, generates predominately exon 7–skipped transcripts (*SMN*Δ*7*) and very low amounts of full-length *SMN* transcripts.

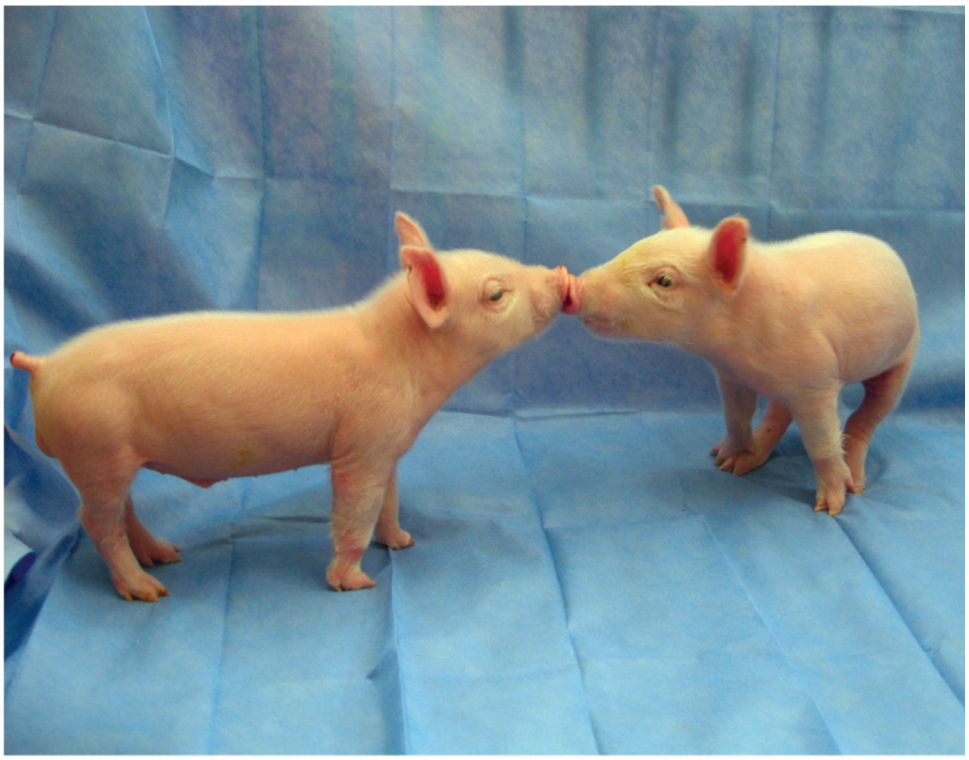

Figure 3.
Founder *SMN*$^{\pm}$ piglets at 10 days of age.

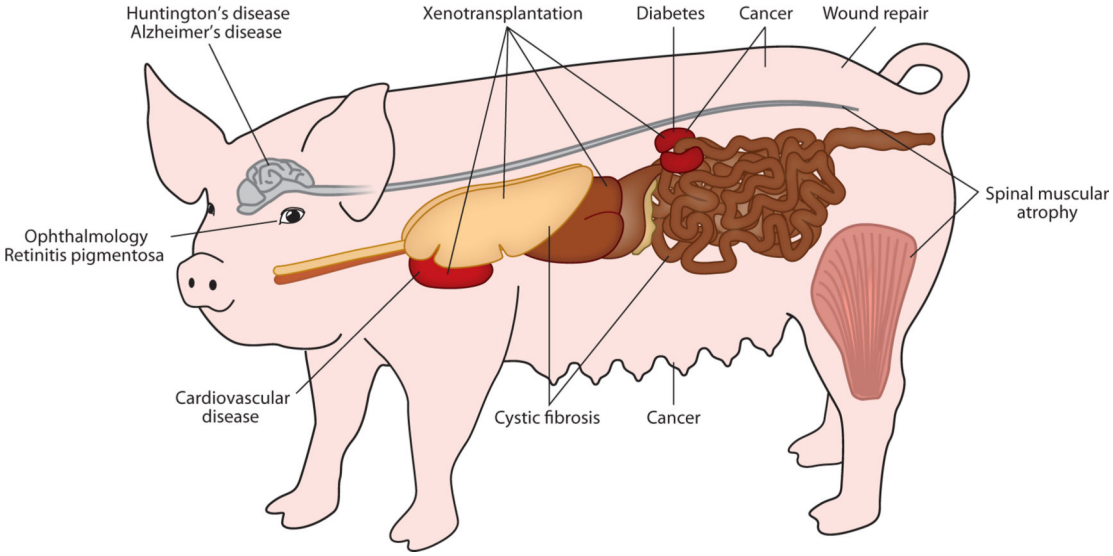

Figure 4.
Organ systems for which genetically engineered pigs have been created.

Table 1

Advantages and disadvantages of various methods of genetic pig modification

Method	Advantages	Disadvantages
Promiclear injection	Can inject large constructs	Little control over the site of integration or the number of copies
Sperm-mediated transfection	Can inject large constructs	Little control over the site of integration or the number of copies
Oocyte transduction	Construct size is limited by the viral system[a]	Little control over the site of integration or the number of copies
Intracytoplasmic sperm injection	Can inject large constructs	Little control over the site of integration or the number of copies
Somatic cell nuclear transfer	Can use multiple methods for inducing genetic modification, e.g., transduction, transfection, zinc finger nucleases, TALENs, transposases. Specific genetic modification can be selected for prior to making the animal.	Low efficiency of cloning. Possible induction of imprinting errors during the cloning process.

[a] Physical limitations of retroviruses, adenoviruses, and adeno-associated viruses is ~4-7 kb, while herpesviruses can accommodate from 20 kb to 150 kb of foreign DNA.