

Genetically modified pigs to model human diseases

Tatiana Flisikowska · Alexander Kind ·
Angelika Schnieke

Received: 16 October 2013 / Accepted: 22 October 2013 / Published online: 15 November 2013
© Institute of Plant Genetics, Polish Academy of Sciences, Poznan 2013

Abstract Genetically modified mice are powerful tools to investigate the molecular basis of many human diseases. Mice are, however, of limited value for preclinical studies, because they differ significantly from humans in size, general physiology, anatomy and lifespan. Considerable efforts are, thus, being made to develop alternative animal models for a range of human diseases. These promise powerful new resources that will aid the development of new diagnostics, medicines and medical procedures. Here, we provide a comprehensive review of genetically modified porcine models described in the scientific literature: various cancers, cystic fibrosis, Duchenne muscular dystrophy, autosomal polycystic kidney disease, Huntington's disease, spinal muscular atrophy, haemophilia A, X-linked severe combined immunodeficiency, retinitis pigmentosa, Stargardt disease, Alzheimer's disease, various forms of diabetes mellitus and cardiovascular diseases.

Keywords Disease model · Gene targeting · Pig · Swine · Transgenic · TALENs · ZFNs

Introduction

Human lifespan is steadily extending and expectations of health and well-being are increasing worldwide. Age- and lifestyle-related diseases such as cancers, diabetes and cardiovascular diseases are already the leading causes of sickness and death, and are predicted to become yet more prevalent. These trends present significant challenges to healthcare budgets and increase pressure to produce new medical strategies.

Preclinical studies are particularly important in the process of developing new diagnostics, drugs and other treatments. The quality and relevance of data obtained at an early stage is critical in predicting clinical effectiveness and safety. Failure of new medicines in late-stage trials is a major waste of resources for pharmaceutical companies and loss of benefit to patients.

The humane treatment of animals is an important priority in biological research. However, as no in vitro models of human whole-body physiology or immune system are available or in immediate prospect, some preclinical animal studies are unavoidable. Ethics requires that those animal trials that must be performed should provide high-quality information directly relevant to human disease. This is best achieved using well-characterised, physiologically relevant animal models.

Mice have come to dominate basic research in mammals because they are convenient, cheap to house and amenable to precise genetic modification. They have provided powerful insights into the molecular basis of many human diseases and enabled proof-of-principle studies for potential biomedical applications. However, mice differ considerably from humans in size, general physiology, anatomy and lifespan, and this restricts their usefulness closer to the clinic. For example, it is not possible to develop surgical procedures or test new medical equipment on the human scale (e.g. endoscopic imaging). There is also growing evidence that many results gained from mice poorly predict findings in humans, for example, in inflammatory conditions (Seok et al. 2013) and several other diseases, as outlined in this review.

Pigs are already a valuable resource for biomedical research. They share many similarities with humans in body size, anatomy, diet and their physiological and pathophysiological responses. They are relatively long lived, which allows longitudinal studies to be carried out in individual animals under conditions that mimic the human patient. Pigs have long been kept as domesticated animals and there are few

T. Flisikowska (✉) · A. Kind · A. Schnieke
Chair of Livestock Biotechnology, Technische Universität München,
Liesel-Beckmann-Str. 1, 85354 Freising, Germany
e-mail: tatiana.adamowicz@wzw.tum.de

additional requirements for their use to model human disease. They adapt well to indoor housing, and designated pathogen-free systems are available where necessary.

The key techniques for generating genetically modified large animals, nuclear transfer (Schnicke et al. 1997) and gene targeting (McCreath et al. 2000), were established more than a decade ago, but it has taken several years for them to be refined sufficiently to produce genetically modified pigs to order. The pace of advance is now increasing with the development of highly specific synthetic endonucleases, TALENs (transcription activator-like effector nucleases), ZFNs (zinc finger nucleases) and RNA-guided endonucleases (Carlson et al. 2012; Hauschild et al. 2011; Flisikowska et al. 2011; Mussolino and Cathomen 2013) and improved porcine genomic sequence data (Groenen et al. 2012). Generating genetically modified pigs takes more time and effort than mice, but the potential benefits for preclinical research and human welfare are substantial. Here, we review the current progress in modelling human diseases. Table 1 summarises the models discussed below.

Cancer

Cancer is the fourth most common cause of human morbidity and mortality worldwide. In 2008, 12.7 million new cases of cancer were diagnosed and the annual incidence is predicted to double by 2030 as more people enter later life (<http://www.cancerresearchuk.org>). Reliable diagnosis at an early stage is key to improving prospects for those affected.

Genetically modified mice have been valuable tools in basic cancer biology, for example, in understanding the molecular mechanisms involved in tumour initiation and progression (reviewed by Frese and Tuveson 2007). However, they are less useful in many preclinical studies. For example, radiation and thermal therapy cannot easily be scaled down to treat mouse-sized tumours (Adam et al. 2008). The evolutionary distance between rodents and humans also leads to problems. Differences in drug metabolism can result in dramatically different responses in human and mice (reviewed by Martignoni et al. 2006).

Prior to our own work, there were three reported attempts to generate pig cancer models by genetic modification. Pigs transgenic for the *v-Ha-ras* oncogene (Yamakawa et al. 1999) showed no phenotype. Pigs that express the Gli2 transcriptional activator in keratinocytes showed basal cell carcinoma-like lesions, but were not fully analysed because they were lost to bacterial infection (McCalla-Martin et al. 2010). The first gene-targeted pigs for cancer were generated by adeno-associated virus-mediated inactivation of *BRCAl* (breast cancer associated gene 1) (Luo et al. 2011). Unfortunately, these did not survive for longer than 18 days, but the same group has recently reported a 2-year-old sow with morphological

changes in the mammary gland (IX Transgenic Animal Research Conference, Lake Tahoe, Tahoe City, California, August 2013).

We are engaged in a programme to generate a series of human cancer and cancer predisposition models in pigs. This was inspired by the fact that similar genetic alterations in a relatively small set of genes are responsible for initiating several human tumour types (Futreal et al. 2004). It should, thus, be possible to replicate different cancers in pigs by combining and activating defined oncogenic mutations in chosen tissues.

We described the first viable, gene-targeted ‘oncopigs’. These carry truncating mutations in the adenomatous polyposis coli (*APC*) gene at sites orthologous to germ line mutations responsible for an inherited form of colorectal cancer, familial adenomatous polyposis (FAP) (Flisikowska et al. 2012). Disease onset in FAP is sooner than the more common sporadic form of colorectal cancer, but the origin and progression of disease are thought to be the same. FAP is characterised by the formation of foci of dysplastic growth of the intestinal epithelium in the colon and rectum that develop to adenomatous polyps, which, if not removed, ultimately progress to adenocarcinoma and metastasis (Fodde and Smits 2001). FAP patients typically develop hundreds of adenomatous polyps in the colon and rectum between puberty and 20 years of age. Polyposis almost inevitably progresses to cancer by the age of 35 to 40 years (Croner et al. 2005).

The examination of a 1-year-old founder animal carrying the mutation *APC*¹³¹¹, orthologous to a severe human FAP mutation *APC*¹³⁰⁹, revealed more than 60 polyps in the colon and rectum that closely resembled human adenomas by a variety of criteria (Flisikowska et al. 2012). The pig phenotype accords with the location and early onset of human FAP, and contrasts with *Apc* mutations in mice. *Apc* mutant mice, such as the widely studied *Min* strain, develop polyps predominantly in the small intestine rather than the colon, and do not show the same disease progression as in humans. Mouse tumours do not invade the intestinal submucosa, rarely develop to adenocarcinoma and do not metastasise (Boivin et al. 2003). We are currently analysing F1 and F2 generation *APC*¹³¹¹ animals to monitor the pattern and time course of polyposis and progression to cancer. Evidence so far indicates that porcine FAP mirrors the development of the human disease.

We have also generated gene targeted pigs carrying a conditionally activated mutant form of the key tumour suppressor p53, *TP53*^{R167H}, orthologous to human *TP53*^{R175H} and mouse *Trp53*^{R172H}, to model Li–Fraumeni syndrome and a wide variety of other cancers (Leuchs et al. 2012). *TP53* is mutated or silenced in the majority of human cancers, so these pigs are vital components in our programme. Evidence so far indicates that the porcine *TP53*^{R167H} mutation confers similar

Table 1 Genetically modified pig models for human diseases

Human disease	Gene and type of modification	Ref.
Cancer		
Different types of cancer	Activated v-H-ras. Viral transgene	Yamakawa et al. (1999)
	Gli2 transcriptional activator, GLI2. Human transgene	McCalla-Martin et al. (2010)
	Tumour protein p53, TP53. Knockout and gene targeted conditionally activated mutation TP53 ^{R167H}	Leuchs et al. (2012)
Breast cancer	Breast cancer 1 early onset, BRCA1. Knockout	Luo et al. (2011)
Familial adenomatous polyposis	Adenomatous polyposis coli, APC. Gene targeted truncating mutations APC ^{L061} and APC ^{I311}	Flisikowska et al. (2012)
Colon cancer	Adenomatous polyposis coli, APC. Knockout	Tan et al. (2013)
Monogenic diseases		
Cystic fibrosis	Cystic fibrosis transmembrane conductance regulator, CFTR. Knockout and gene targeted CFTR ^{ΔF508} mutation	Rogers et al. (2008a, b)
Duchenne muscular dystrophy	Dystrophin, DMD. Gene targeted exon 52 deletion	Klymiuk et al. (2013)
Autosomal dominant polycystic kidney disease	Polycystic kidney disease type 2 gene, PKD2. Porcine transgene	He et al. (2013)
	Proto-oncogene <i>CMYC</i> . Porcine transgene	Ye et al. (2013)
Huntington's disease	Huntingtin, HTT. Human mutant transgene	Uchida et al. (2001)
	Huntingtin, HTT. Human 208 aa N-terminal region transgene	Yang et al. (2010)
	Huntingtin, HTT. Human 548 aa N-terminal region transgene	Baxa et al. (2013)
Spinal muscle atrophy	Survival of motor neuron 1 telomeric, SMN1. Knockout	Lorson et al. (2011)
Haemophilia A	Coagulation factor VIII, F8. Knockout	Kashiwakura et al. (2012)
X-linked severe combined immunodeficiency	Interleukin 2 receptor, γ , IL2RG. Knockout	Suzuki et al. (2012)
Retinitis pigmentosa	Rhodopsin, RHO. Porcine mutant RHO ^{P347L} transgene	Petters et al. (1997)
	Rhodopsin, RHO. Human mutant RHO ^{P23H} transgene	Ross et al. (2012)
	Elongation of very long chain fatty acids-4, ELOVL4. Human mutant ELOVL4 ^{5bbDel} transgene	Sommer et al. (2011)
Stargardt disease-3	Elongation of very long chain fatty acids-4, ELOVL4. Human mutant ELOV4 ^{Y270} transgene	Sommer et al. (2011)
Multifactorial diseases		
Alzheimer's disease	Amyloid precursor protein, APP. Human mutant APP ^{sw} transgene	Kragh et al. (2009)
Diabetes type 2	Glucose-dependent insulinotropic polypeptide, GIPR. Human mutant GIPR ^{dn} transgene	Renner et al. (2010)
Permanent neonatal diabetes mellitus	Insulin, INS. Porcine mutant INS ^{C94Y} transgene	Renner et al. (2013)
Diabetes type 3 (MODY)	Hepatocyte nuclear factor 1 alpha, HNF-1 α . Human mutant HNF-1 α ^{P291fsinsC}	Umeyama et al. (2009)
Cardiovascular disease	Low-density lipoprotein receptor, LDLR. Knockout	Carlson et al. (2012)
	Proprotein convertase subtilisin/kexin type 9, PCSK9. Human mutant PCSK9 ^{D37Y} transgene	Al-Mashhadi et al. (2013)
	Endothelial nitric oxide synthase, NOS3. Porcine transgene	Hao et al. (2006)
	Catalase, CAT. Human transgene	Whyte et al. (2011)
	Peroxisome proliferator-activated receptor- γ , PPAR-G. Knockout	Yang et al. (2011)
	Apolipoprotein C3, APOC3. Human transgene	Wei et al. (2012)

changes as in humans and mice. Mutant p53-R167H protein accumulates in affected cells, indicating the failure of normal p53 degradation (Midgley and Lane 1997), and also confers resistance to the chemotherapeutic drug doxorubicin (Leuchs et al. 2012). Piglets carrying the latent TP53^{R175H} allele are viable and healthy. These are only the first steps; our group and others are generating more cancer-related mutations (e.g. Tan et al. 2013). A powerful toolkit to model diverse cancers is being created.

Monogenic diseases

Cystic fibrosis

Cystic fibrosis (CF) is an incurable chronic disease that primarily affects the lungs, but also other organs, including the pancreas, liver and intestine. CF is the most common autosomal recessive human genetic disorder, and is caused by mutations in the *CFTR* (cystic fibrosis transmembrane

conductance regulator) gene (Riordan et al. 1989). Several mutant mouse strains have been generated, including *Cftr* null alleles and mutations that replicate known human lesions (reviewed by Bragonzi 2010), but these fail to recapitulate the natural progression of the disease and the critical lung and pancreatic pathologies that afflict human patients (Guilbault et al. 2007).

Pig and human lungs share many anatomical, histological, biochemical and physiological features (Rogers et al. 2008a). Gene targeting using adeno-associated virus and nuclear transfer has been used to produce pigs with the most common CF-associated mutation, *CFTR* Δ F508, and also *CFTR* knockout (Rogers et al. 2008b). *CFTR*-deficient newborn piglets develop defects similar to newborn human CF patients; meconium ileus, exocrine pancreatic destruction and hepatic changes consistent with early focal biliary cirrhosis. Within a few months of birth, these pigs spontaneously develop lung disease, including inflammation, remodelling, mucus accumulation and infection, a set of clinical indications found in human patients, but absent in mice (Stoltz et al. 2013). Subsequent studies have also revealed that *CFTR* gene disruption directly affects nervous system function. Newborn CF pigs showed features of axonal and demyelinating neuropathy consistent with findings in human CF (Reznikov et al. 2013). The CF model is, perhaps, the most advanced of the porcine disease models and is already contributing to improved analysis, treatment and therapy.

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a lethal X-linked muscular disorder that affects males (1 in 3,500 male births), causing progressive muscle weakness, wasting, disability and eventual death due to respiratory or heart failure (Spurney 2011). The first symptoms are apparent before the age of 5 years. The disease is caused by frameshift mutations in the *DMD* gene, leading to loss of function of the essential muscle protein dystrophin (Hoffman et al. 1987). Most mutations occur in two hot spot regions of *DMD*, exons 3–7 and exons 45–55 (Koenig et al. 1989).

Several mouse models have been created to replicate DMD, but these show a much milder phenotype than human DMD. Other naturally occurring mutations have been described in dogs and cats. The canine model resembles human DMD but is caused by a mutation that does not occur in human patients. The feline model does not resemble human DMD pathologically (Nakamura and Takeda 2011).

Pigs have recently been described with a targeted deletion of *DMD* exon 52, a frequent mutation in human patients (Lu et al. 2011). These animals show pathological and functional hallmarks of human DMD, such as the absence of dystrophin in skeletal muscles, increased serum creatine kinase levels, progressive dystrophic changes of skeletal muscles, impaired mobility, muscle weakness and a lifespan of 3 months.

However, unlike humans, some of these pigs died shortly after birth. Interestingly, these piglets have already provided new insight into early changes associated with physiological derangements of dystrophic muscle, by highlighting the possible importance of *CCL2* (CC chemokine ligand 2) down regulation. *CCL2* is a ligand of the CC chemokine receptor 2, which is associated with inflammation and muscle regeneration (Lu et al. 2011). Older DMD pigs could serve as a model for developing and testing new therapeutic strategies for DMD, such as exon skipping, gene and stem cell therapy.

Autosomal polycystic kidney disease

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common inherited diseases (1 in 400 to 1 in 1,000 people worldwide). ADPKD is characterised by the progressive formation and enlargement of cysts predominantly in the kidney, but also in other organs (e.g. liver, pancreas, spleen) (Gabow 1993). Approximately 50 % of affected patients develop renal failure and require kidney replacement by the age of 60 years (Grantham 2008). Mutations in *PKD1* and *PDK2* (polycystic kidney disease) are responsible, with *PDK1* accounting for ~85 % of all cases (Pignatelli et al. 1992; Kimberling et al. 1993; Mochizuki et al. 1996).

Several different mice models have been generated to study the aetiology of ADPKD and a number of hypotheses have emerged. These are: the two-hit hypothesis (an inherited germ-line mutation followed by a somatic mutation) (Lu et al. 1997), the third-hit hypothesis (ischaemia/reperfusion, nephrotoxic injury and compensatory hypertrophy) (Takakura et al. 2009) and the threshold effect hypothesis (hypomorphic or overexpressed alleles) (Pritchard et al. 2000). ADPKD is, however, a chronic disease, and the short lifespan of mice precludes long-term studies.

The pig urological system shares many anatomical, histological and physiological features with that of humans (Zaidi et al. 1998; Dekel et al. 2003). Recently, the generation of two minipig lines based on the threshold effect hypothesis have been reported (He et al. 2013; Ye et al. 2013). The first line overexpressed *PDK2* under the CMV (cytomegalovirus) promoter. The second line overexpressed the proto-oncogene *CMYC* specifically in the kidney (*CMYC* is up regulated in cystic kidneys) (Cowley et al. 1987; Lanoix et al. 1996). To date, neither transgenic line has been reported to show significant alteration in kidney function, and the authors predict that two to three years may be required for renal dysfunction to manifest.

Huntington's disease

Huntington's disease (HD) is a neurodegenerative genetic disorder involving the progressive breakdown of brain neurons, leading to impairment of motor, emotional and cognitive functions. The underlying genetic defect is the expansion of a

trinucleotide (CAG) repeat stretch in the first exon of the *HTT* (huntingtin) gene (Gusella et al. 1983). These are expressed as a series of glutamine residues, or polyglutamine tract. The severity of the disease varies with the number of CAG repeats, ranging from 36 (mild) to over 40 units (severe).

A variety of species have been used to model HD, including invertebrates (*D. melanogaster*, *C. elegans*), mice, sheep, monkeys and, most recently, pigs (reviewed by Zuccato et al. 2010). However, it has proved to be a difficult task. Transgenic mice fail to mimic the most important characteristic of HD, the neuronal apoptosis that underlies neurodegeneration. This is believed to be a consequence of differences in human and rodent brain anatomy and *HTT* gene function (Li and Li 2012). Transgenic sheep (Jacobsen et al. 2010) and rhesus monkeys (Yang et al. 2008) have been generated as alternatives, but neither showed symptoms resembling HD.

Three lines of transgenic pigs have been produced to study HD. The first was generated by the microinjection of a mutant *HTT* transgene (1,100 amino acids), but no phenotype was observed (Uchida et al. 2001). The second HD pig line expressed the first (N-terminal) 208 amino acids of human *HTT* with an expanded (105 residues) polyglutamine tract under the control of the ubiquitous cytomegalovirus enhancer/chicken beta-actin promoter (Yang et al. 2010). Some of these pigs showed chorea-like movement, a common symptom of HD. Furthermore, unlike mice that expressed the same transgene, the pigs showed apoptosis of brain neurons typical of human HD. Long-term studies were, however, not possible because of significant postnatal death. More recently, Baxa et al. (2013) reported pigs carrying a transgene encoding the first 548 amino acids of *HTT* with a 124-residue polyglutamine tract directed by the human *HTT* promoter. These showed normal development after birth. Further analysis at 16 months did not reveal the formation of aggregates in the brain, and no obvious signs of abnormal movement up to 40 months. They did, however, show reduced levels of an indicator of HD, the signalling protein DARPP32, in the neostriatum, which is the brain region most affected in HD. Whether these pigs provide a representative model for HD remains to be seen.

Spinal muscular atrophy

Spinal muscular atrophy (SMA) is the leading genetic cause of infant death. SMA is characterised by the degeneration of spinal cord motor neurons, leading to muscle wasting and impaired mobility. It is an autosomal recessive disorder caused by defects in the *SMN1* gene (survival of motor neuron 1, telomeric). Unusual amongst mammals, humans also have several copies of a nearly identical gene, *SMN2*. Despite its sequence identity, *SMN2* does not protect against defects in *SMN1* because it is expressed as an RNA splice variant and an unstable truncated protein (Lorson et al. 1999). *SMN2* does,

however, act as a disease modifier, with the *SMN2* gene copy number being roughly inversely related to SMA severity.

Several mouse models have been developed to replicate SMA, but these exhibit a rapid, progressive development of the disease that is too severe to allow the testing of potential therapeutic agents (Beebe et al. 2012).

To generate a pig model for SMA that properly models the human disease, it is necessary not only to inactivate the porcine *SMN1* gene, but also to introduce the human *SMN2* gene. This is because the inactivation of *SMN1* in non-human mammals is embryonically lethal (Schrank et al. 1997; Hsieh-Li et al. 2000; Monani et al. 1999). Lorson et al. (2011) have generated heterozygous *SMN1* knockout pigs by nuclear transfer as the first step towards modelling SMA. Piglets showed a normal phenotype after birth. They also demonstrated that human *SMN2* is spliced in the human manner in porcine cells, suggesting that a porcine model of SMA is feasible.

Haemophilia A

Haemophilia A is the most common form of haemophilia. The most obvious symptom is bleeding diathesis, such as bleeding in the brain and harmful bleeding in joints and muscles. Haemophilia A is an inherited X-linked disorder that affects 1 in 5,000 male live births (Mannucci and Tuddenham 2001). It is caused by a variety of different mutations in the *F8* (coagulation factor VIII) gene that result in dysfunction or loss of factor VIII protein.

Transgenic mice carrying a disrupted *F8* gene have been used to investigate methods of gene therapy and evaluate factor VIII variants used in replacement therapy (Bi et al. 1995). However, these mice rarely exhibit spontaneous bleeding into the muscles and joints under standard breed conditions, limiting their clinical usefulness. The difference in phenotype arises from differences in the mouse and human coagulation and immune systems. For many years, the most representative animal models have been dogs with natural mutations in *F8*.

The porcine blood coagulation system is very similar to that in humans, and porcine factor VIII has been successfully used to treat haemophilia A patients (Morrison et al. 1993; Barrow and Lollar 2006; Toschi 2010). Pigs with targeted disruption of *F8* have recently been described (Kashiwakura et al. 2012). These animals show a persistent bleeding phenotype and died within two days if not treated. The infusion of human factor VIII reduced bleeding but piglets required the inhibition of anti-human factor VIII antibodies to fully stop the bleeding. This pig model promises to be useful in studying the management of bleeding disorders and the development of new therapeutics, such as factor VIII variants with slower clearance rate.

X-linked severe combined immunodeficiency

Severe combined immunodeficiency (SCID) is a group of inherited immunodeficiency disorders that affect humans, and also occur naturally in horses and dogs. SCID patients often suffer from severe bacterial, viral and fungal infections before 3 months of age, commonly followed by lung inflammation. SCID individuals also suffer diarrhoea, sepsis and middle ear infections (Vickers 2009).

SCID has been linked to defects in at least 13 human autosomal genes, including *ADA* (adenosine deaminase) and *JAK3* (Janus kinase 3). However, the most common form is X-linked (X-SCID) and caused by mutations in *IL2RG* (interleukin 2 receptor, γ) that lead to loss of T and NK (natural killer) cells and impairment of B cell function (Noguchi et al. 2008; Leonard 1996; Fischer et al. 1997).

Il2rg-deficient SCID mice are very widely used, for example, as recipients for allogeneic and xenogeneic tissue transplantation (Leblond et al. 1997), and have made major contributions to the understanding of the immunodeficiency mechanism, lymphocyte development and DNA repair. More recently, SCID rats have been generated by ZFN technology (Mashimo et al. 2010, 2012). SCID pigs carrying targeted disruption of *IL2RG* have also been generated (Suzuki et al. 2012). *IL2RG*-deficient boars were athymic and exhibited markedly impaired production of T and NK cells, strongly resembling human SCID. Because their body size resembles humans, SCID pigs will be a valuable new means of modelling human patients in organ, tissue and cell transplantation experiments.

Retinitis pigmentosa

Retinitis pigmentosa (RP) is an inherited retinal disease characterised by the early and rapid loss of light-sensitive photoreceptor neurons, leading to progressive loss of peripheral vision and eventual loss of central vision. Mutations in a variety of photoreceptor or pigment epithelium-specific genes can result in retinal degeneration, but the majority of RP cases are due to mutation in the *RHO* (rhodopsin) gene.

Mice carrying different mutations in *Rho* have been generated (Olsson et al. 1992; Li et al. 1996; Humphries et al. 1997), but the structure of the rodent eye and retina differs from humans, and these models do not mimic the human disease.

The porcine eye more closely resembles humans in size, anatomy and the structure of the optic nerve and retina. There are two reports of *RHO* transgenic pigs. Transgenic pigs carrying mutant porcine *RHO*^{P347L} were generated by pronuclear microinjection (Petters et al. 1997) and pigs carrying mutant human *RHO*^{P23H} were generated by nuclear transfer (Ross et al. 2012). Both lines showed early and severe loss of

photoreceptors and progressive dysfunction of photoreceptor cones.

Stargardt disease-3

Stargardt disease type 3 is a genetic eye disease characterised by early macular degeneration and decreased visual acuity (Edwards et al. 1999). It is associated with truncating mutations in *ELOVL4* (elongation of very long chain fatty acids 4). The eyes of pigs, unlike mice, have a macula and are a better model for human macular degeneration. Transgenic pigs carrying two different *ELOVL4* truncating mutations have been generated (Sommer et al. 2011). These displayed photoreceptor loss, disorganisation of inner and outer segments, and electroretinography responses.

Multifactorial diseases

Alzheimer's disease

Alzheimer's disease (AD) is a multifactorial, progressive brain disorder characterised by loss of memory and disorientation. The cause of the disease is still not fully understood, but mutations in several candidate genes have been suggested as being responsible. These are missense mutations in *APP* (amyloid precursor protein), *PSEN1* and *PSEN2* (presenilin 1 and 2), which are associated with increased production of beta amyloid protein fragments (Scheuner et al. 1996). The accumulation of abnormally folded amyloid proteins initiates a pathogenic process that leads to the formation of intraneuronal neurofibrillary tangles of the microtubule-binding protein tau and, eventually, loss of neurons (Hardy and Selkoe 2002).

Transgenic mice have been generated that express mutant forms of human *APP* (e.g. V717F, L670N, M671L), identified as tightly associated with AD. These showed age-related development of pathological deposits in the brain that resembled AD senile plaques (Games et al. 1995; Hsiao et al. 1996), but neurofibrillary tangles were absent, and the massive neuronal loss observed in humans was not evident (Takeuchi et al. 2000). Kragh et al. (2009) have generated minipigs containing a human *APP* transgene bearing the so-called Swedish mutation, *APP*^{sw}, with expression directed by the human platelet-derived growth factor-beta promoter. Analysis of founder pigs produced by nuclear transfer showed that they carried single-copy transgenes with strong, promoter-specific expression of mutant APP protein in the brain. These animals are 5 years old at the time of writing, but no disease phenotype has yet been reported. It is not clear how long it will be necessary to wait, nor how the aging process in pigs relates to humans.

Diabetes mellitus

Diabetes is a group of metabolic disorders characterised by hyperglycaemia. Type 1 diabetes is autoimmune in origin. The destruction of pancreatic islet beta cells decreases the amount of insulin produced, leading to loss of blood glucose regulation. Type 2 diabetes arises from a chronic decrease in the response to normal levels of insulin, leading to progressive beta cell dysfunction and compensatory hyperinsulinaemia. Type 2 diabetes has genetic risk components, but is strongly influenced by lifestyle factors. Type 3 diabetes, also known as maturity onset diabetes of the young (MODY), is an early-onset form of type 2 diabetes with an autosomal dominant pattern of inheritance (Yamagata et al. 1996, 1998).

Several different rodent models are currently used to study diabetes. These have been generated by introducing structural or functional modifications in candidate genes, e.g. *Insr* (insulin receptor), *Ppar-a*, *-b* and *-g* (peroxisome proliferator activated receptor $-\alpha$, $-\beta$, $-\gamma$) (reviewed by Plum et al. 2005), or random mutagenesis (reviewed by Aigner et al. 2008). Rodent models are invaluable tools in understanding the molecular basis of insulin action in glucose metabolism, insulin receptor signalling and pathways, lipid metabolism and adipocyte biology, but they fail to replicate important aspects of the human disease state, notably, insulin resistance and obesity. Moreover, important diabetes-associated conditions often seen in human patients, such as atherosclerosis and hyperlipidaemia, do not occur in rodent models. These can, however, be induced by the modification of additional genes such as *ApoE* (apolipoprotein E) (Zhang et al. 1992), leptin and *Ldlr* (low-density lipoprotein receptor) (Hasty et al. 2001), see also the following section on “Cardiovascular diseases”.

Two key gut hormones, gastric inhibitory polypeptide (also known as glucose-dependent insulinotropic polypeptide; GIP) and glucagon-like peptide-1 (GLP-1), are targets for the treatment of type 2 diabetes. They are secreted after oral glucose administration and increased insulin secretion in response to hyperglycaemia. In patients with type 2 diabetes, the insulinotropic response to GIP is significantly reduced, suggesting that altered GIP action might be involved in the early pathogenesis of the condition (Nauck et al. 2004). This finding led to the generation of a pig model that mimics the essential features of type 2 diabetes. Transgenic pigs expressing a dominant-negative mutant form of the human GIP receptor (*GIPR^{dn}*) under the control of the rat insulin (*Ins2*) promoter in pancreatic islets have been generated using a lentiviral vector (Renner et al. 2010). At an early age (11 weeks), these pigs showed reduced tolerance for oral glucose loads as a result of delayed insulin release. They also displayed reduced beta cell mass as they age. Metabolomic studies of *GIPR^{dn}* pigs revealed changes in the plasma concentration of seven amino acids (Phe, Orn, Val, Leu, His, Arg and Tyr) compared to control animals. A decreased

concentration of specific lipids such as sphingomyelins, diacylglycerols and ether phospholipids was also observed in the plasma of 5-month-old *GIPR^{dn}* transgenic pigs. These metabolites represent candidate biomarkers that may serve as indicators of early-stage type 2 diabetes (Renner et al. 2012).

The same group recently reported the generation of a porcine model of another type of diabetes, permanent neonatal diabetes mellitus based on the expression of a mutant (C94Y) insulin transgene in pancreatic beta cells. At 4.5 months old, these pigs had reduced body weight, decreased beta cell mass and reduced fasting insulin levels. They also showed cataract development as early as 8 days after birth (Renner et al. 2013).

Another transgenic pig line has been produced to model MODY disease (Umeyama et al. 2009). The MODY pigs carried a mutant form of the *HNF1A* (homeobox nuclear factor 1 α) gene, which is known to cause the human disease. The transgenic pigs that survived for 20–196 days exhibited increased blood glucose level and pancreas abnormalities.

Cardiovascular diseases

Cardiovascular diseases (CVD) constitute the leading cause of death worldwide, worse by a substantial margin than the total of all infectious diseases. CVD usually affects older adults but precursor conditions begin earlier, notably atherosclerosis. Atherosclerosis is a chronic inflammatory condition that involves the accumulation of lipids, thickening of arterial walls and the formation of plaques that chronically expand and occlude blood flow. Plaques are liable to rupture and form thrombi precipitating acute, life-threatening episodes such as cardiac infarction and stroke.

Modelling atherosclerosis offers a useful means of investigating treatments and preventions such as lifestyle changes, low-density lipoprotein cholesterol-lowering drugs and other therapies. Pigs, like humans, develop atherosclerosis in response to an atherogenic diet (Dixon et al. 1999), while normal mice are resistant to dietary cholesterol. Genetically modified animals, however, provide greater reproducibility and consistency between experimental treatments than purely dietary-induced CVD models. They can also be used to investigate genetic risk factors, such as alleles identified in human epidemiological studies, and, so, inform personalised medicine.

In humans, mutations in genes involved in lipoprotein metabolism, notably the low-density lipoprotein receptor (*LDLR*) and apolipoprotein E (*APOE*), lead to familial hypercholesterolaemia and atherosclerosis. *Ldlr*-deficient and *ApoE*-deficient mice have been widely studied. *Ldlr*-deficient mice fed a normal diet show a moderate increase of plasma cholesterol level and slowly develop atherosclerosis, and on a high-fat diet, they develop hypercholesterolaemia and atherosclerotic lesions (Ishibashi et al. 1993; Knowles and Maeda 2000; Bentzon and Falk 2010). *ApoE*-deficient mice develop

a wide spectrum of lesions observed during atherogenesis, such as elevated plasma cholesterol and atherosclerosis in the aorta (Zhang et al. 1992; Plump et al. 1999). These mouse models are valuable for investigating atherogenic mechanisms, but have limited applicability in preclinical studies. One problem is their failure to model plaque rupture and thrombosis. It is also difficult to investigate human lifestyle risk factors, especially food, because mice do not accept a human diet.

The pig is well suited to the study of cardiovascular diseases, and enables studies that are difficult or impossible in mice. The pig cardio- and cerebrovascular system has a similar anatomy to humans. Porcine blood parameters and vessel size are similar, allowing real-time measurements (e.g. blood flow, temperature) using human medical devices. This allows the investigation of new technologies such as imaging to visualise the location, extent and inflammation status of atherosclerotic plaques. Porcine metabolic values also resemble humans, and pigs readily accept a human diet.

Much current work towards modelling CVD in pigs is focussed on the *LDLR* gene. Minipigs with both mono- and biallelic inactivation of *LDLR* have been produced using TALENs (Carlson et al. 2012). A company website also reports that *LDLR*-deficient pigs show high plasma cholesterol and develop atherosclerotic lesions even when fed a vegetarian, cholesterol-free diet (<http://exemplargenetics.com/ldlr/>).

Other genes have also been modified in pigs for CVD studies. Natural mutations in *PCSK9* (proprotein convertase subtilisin/kexin type 9) are responsible for a severe form of human hypercholesterolaemia (Soutar 2011). One role of *PCSK9* is to regulate LDL by binding hepatic LDLRs and targeting them for lysosomal degradation (Mousavi et al. 2009). The D374Y gain-of-function mutation increases binding affinity and causes a severe form of hypercholesterolaemia (Soutar 2011). Minipigs that overexpress human *PCSK9*^{D374Y} have been generated. As in humans, these pigs developed severe hypercholesterolaemia when fed a high-fat, high-cholesterol diet, and also showed human-like progressive atherosclerosis of the heart and great vessels (Al-Mashhadi et al. 2013).

Endothelial nitric oxide synthase (eNOS), which catalyses the production of nitric oxide (NO) in the inner lining of blood vessels, plays a key role in cardiovascular regulation. Transgenic pigs that overexpress eNOS were generated several years ago (Hao et al. 2006), but there has been no comprehensive description of the phenotype.

Hydrogen peroxide (H₂O₂) is also involved in the regulation of NO signalling in vascular endothelium. For example, H₂O₂ stimulates eNOS expression in response to increased oxidation stress (e.g. during exercise training) (Thomas et al. 2007). However, its role in CVD is not fully understood. The enzyme catalase tightly regulates H₂O₂ levels in vascular

tissues by decomposing it to oxygen and water. Transgenic mice that overexpress human catalase in endothelium have been generated, but no effect on endothelial eNOS expression was evident (Lauer et al. 2005). Transgenic minipigs that express endothelial catalase have been generated to investigate the role of endothelium-derived H₂O₂ signalling and potentially provide a CVD model (Whyte et al. 2011).

Peroxisome proliferator-activated receptor- γ (PPAR- γ) (see also the section on “Diabetes mellitus”) has been identified as a therapeutic target for CVD. PPAR- γ is important in adipogenesis and is expressed in adipocytes and many other cell types, including vascular smooth muscle cells, endothelial cells, macrophages and cardiomyocytes (Jiang et al. 1998; Ricote et al. 1998; Benson et al. 2000). Thiazolidinediones are high-affinity synthetic ligands that act as PPAR- γ agonists. Mouse studies showed that they reduced atherosclerosis, but they were found to increase the risk of heart ischaemia in human patients (Nissen and Wolski 2007). In 2011, Yang et al. (2011) reported the inactivation of *PPAR-G* in viable pigs using a zinc finger nuclease and nuclear transfer, providing a new tool to study the role of PPAR- γ in cardiovascular disease.

Lipid accumulation is a major factor in atherosclerotic plaque formation. Apolipoprotein CIII is a major regulator of plasma triglyceride metabolism and closely associated with hypertriglyceridaemia in patients with metabolic syndrome (Cohn et al. 2004). Wei et al. (2012) reported minipigs that overexpress the human *APOC3* gene as a model for studying hypertriglyceridaemia and atherosclerosis. These animals show raised plasma triglyceride levels and reduced lipoprotein lipase activity (Wei et al. 2012).

CVD is commonly comorbid with other life-threatening and debilitating conditions, such as diabetes (Parekh and Barton 2010), and these are important considerations when choosing appropriate treatments. Further characterisation of CVD models will reveal if comorbidities emerge spontaneously, or whether it is necessary to generate multiple-mutant animals. As we have described, a diverse range of pig disease models are now being generated. The growing resource of pig strains will, thus, allow mutations to be combined by breeding so that human diseases can be modelled in an ever more realistic manner.

Conclusions and outlook

We are now in the fortunate position that the genetic basis of several serious human diseases is reasonably well understood, allowing many candidate genetic lesions to be replicated in pigs. Pigs are unlikely to supplant mice as subjects for basic research, but are already providing powerful complementary resources for translational studies. This role can be summarised by the slogan “bridging the gap between

laboratory and bedside”. Of course, generating new animal strains is only part of the story, as each new potential model will require considerable characterisation. During this process, it will become clear which diseases and disease predispositions can be most accurately modelled in pigs and efforts directed accordingly. Nevertheless, it is already evident that several new porcine strains represent a major advance on previously available animal models.

The toolbox available for livestock biotechnology is steadily improving, but some key items on the technical ‘wish list’ are still outstanding. One is a reliable means of activating or deactivating modified alleles, in particular, tissues in whole animals. Conditional gene expression based on Cre/loxP and other site-specific recombination systems is well established in mice (e.g. Hingorani et al. 2005), but has not yet been extended to large animals. Mouse geneticists have available a wide range of engineered strains that express Cre under diverse tissue-specific and inducible promoters. The effort required to assemble a set of transgenic pigs expressing Cre recombinase in different tissues would be substantial but worthwhile given their usefulness across a range of research fields. As the field of porcine biomedicine becomes ever larger, there is much to be gained by coordinating research efforts.

References

- Adam M, Bayer C, Henke J, Grosu A, Molls M, Nieder C (2008) Tirapazamine plus cisplatin and irradiation in a mouse model: improved tumor control at the cost of increased toxicity. *J Cancer Res Clin Oncol* 134:137–146
- Aigner B, Rathkolb B, Herbach N, Hrabé de Angelis M, Wanke R, Wolf E (2008) Diabetes models by screen for hyperglycemia in phenotype-driven ENU mouse mutagenesis projects. *Am J Physiol Endocrinol Metab* 294:E232–E240
- Al-Mashhadi RH, Sørensen CB, Kragh PM, Christoffersen C, Mortensen MB, Tolbod LP et al (2013) Familial hypercholesterolemia and atherosclerosis in cloned minipigs created by DNA transposition of a human PCSK9 gain-of-function mutant. *Sci Transl Med* 5:166ra1. doi:10.1126/scitranslmed.3004853
- Barrow RT, Lollar P (2006) Neutralization of antifactor VIII inhibitors by recombinant porcine factor VIII. *J Thromb Haemost* 4:2223–2229
- Baxa M, Hruska-Plochan M, Juhas S, Vodicka P, Pavlok A, Juhasova J et al (2013) A transgenic minipig model of Huntington’s disease. *J Huntingtons Dis* 2:47–68
- Bebee TW, Dominguez CE, Chandler DS (2012) Mouse models of SMA: tools for disease characterization and therapeutic development. *Hum Genet* 131:1277–1293
- Benson S, Wu J, Padmanabhan S, Kurtz TW, Pershadsingh HA (2000) Peroxisome proliferator-activated receptor (PPAR)-gamma expression in human vascular smooth muscle cells: inhibition of growth, migration, and c-fos expression by the peroxisome proliferator-activated receptor (PPAR)-gamma activator troglitazone. *Am J Hypertens* 13:74–82
- Bentzon JF, Falk E (2010) Atherosclerotic lesions in mouse and man: is it the same disease? *Curr Opin Lipidol* 21:434–440
- Bi L, Lawler AM, Antonarakis SE, High KA, Gearhart JD, Kazazian HH Jr (1995) Targeted disruption of the mouse factor VIII gene produces a model of haemophilia A. *Nat Genet* 10:119–121
- Boivin GP, Washington K, Yang K, Ward JM, Pretlow TP, Russell R et al (2003) Pathology of mouse models of intestinal cancer: consensus report and recommendations. *Gastroenterology* 124:762–777
- Bragonzi A (2010) Murine models of acute and chronic lung infection with cystic fibrosis pathogens. *Int J Med Microbiol* 300:584–593
- Carlson DF, Tan W, Lillico SG, Stverakova D, Proudfoot C, Christian M et al (2012) Efficient TALEN-mediated gene knockout in livestock. *Proc Natl Acad Sci U S A* 109:17382–17387
- Cohn JS, Patterson BW, Uffelman KD, Davignon J, Steiner G (2004) Rate of production of plasma and very-low-density lipoprotein (VLDL) apolipoprotein C-III is strongly related to the concentration and level of production of VLDL triglyceride in male subjects with different body weights and levels of insulin sensitivity. *J Clin Endocrinol Metab* 89:3949–3955
- Cowley BD Jr, Sardo FL Jr, Grantham JJ, Calvet JP (1987) Elevated c-myc protooncogene expression in autosomal recessive polycystic kidney disease. *Proc Natl Acad Sci U S A* 84:8394–8398
- Croner RS, Brueckl WM, Reingruber B, Hohenberger W, Guenther K (2005) Age and manifestation related symptoms in familial adenomatous polyposis. *BMC Cancer* 5:24
- Dekel B, Burakova T, Arditti FD, Reich-Zeliger S, Milstein O, Aviel-Ronen S et al (2003) Human and porcine early kidney precursors as a new source for transplantation. *Nat Med* 9:53–60
- Dixon JL, Stoops JD, Parker JL, Laughlin MH, Weisman GA, Sturek M (1999) Dyslipidemia and vascular dysfunction in diabetic pigs fed an atherogenic diet. *Arterioscler Thromb Vasc Biol* 19:2981–2992
- Edwards AO, Miedziak A, Vrabec T, Verhoeven J, Acott TS, Weleber RG et al (1999) Autosomal dominant Stargardt-like macular dystrophy: I. Clinical characterization, longitudinal follow-up, and evidence for a common ancestry in families linked to chromosome 6q14. *Am J Ophthalmol* 127:426–435
- Fischer A, Cavazzana-Calvo M, De Saint Basile G, DeVillartay JP, Di Santo JP, Hivroz C et al (1997) Naturally occurring primary deficiencies of the immune system. *Annu Rev Immunol* 15:93–124
- Flisikowska T, Thorey IS, Offner S, Ros F, Lifke V, Zeitler B et al (2011) Efficient immunoglobulin gene disruption and targeted replacement in rabbit using zinc finger nucleases. *PLoS One* 6:e21045. doi:10.1371/journal.pone.0021045
- Flisikowska T, Merkl C, Landmann M, Eser S, Rezaei N, Cui X et al (2012) A porcine model of familial adenomatous polyposis. *Gastroenterology* 143:1173–1175.e7
- Fodde R, Smits R (2001) Disease model: familial adenomatous polyposis. *Trends Mol Med* 7:369–373
- Frese KK, Tuveson DA (2007) Maximizing mouse cancer models. *Nat Rev Cancer* 7:645–658
- Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R et al (2004) A census of human cancer genes. *Nat Rev Cancer* 4:177–183
- Gabow PA (1993) Autosomal dominant polycystic kidney disease. *Am J Kidney Dis* 22:511–512
- Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C et al (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature* 373:523–527
- Grantham JJ (2008) Clinical practice. Autosomal dominant polycystic kidney disease. *N Engl J Med* 359:1477–1485
- Groenen MA, Archibald AL, Uenishi H, Tuggle CK, Takeuchi Y, Rothschild MF et al (2012) Analyses of pig genomes provide insight into porcine demography and evolution. *Nature* 491:393–398
- Guilbault C, Saeed Z, Downey GP, Radzioch D (2007) Cystic fibrosis mouse models. *Am J Respir Cell Mol Biol* 36:1–7
- Gusella JF, Wexler NS, Conneally PM, Naylor SL, Anderson MA, Tanzi RE et al (1983) A polymorphic DNA marker genetically linked to Huntington’s disease. *Nature* 306:234–238

- Hao YH, Yong HY, Murphy CN, Wax D, Samuel M, Rieke A et al (2006) Production of endothelial nitric oxide synthase (eNOS) over-expressing piglets. *Transgenic Res* 15:739–750
- Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297:353–356
- Hasty AH, Shimano H, Osuga J, Namatame I, Takahashi A, Yahagi N et al (2001) Severe hypercholesterolemia, hypertriglyceridemia, and atherosclerosis in mice lacking both leptin and the low density lipoprotein receptor. *J Biol Chem* 276:37402–37408
- Hauschild J, Petersen B, Santiago Y, Queisser AL, Carnwath JW, Lucas-Hahn A et al (2011) Efficient generation of a biallelic knockout in pigs using zinc-finger nucleases. *Proc Natl Acad Sci U S A* 108:12013–12017
- He J, Ye J, Li Q, Feng Y, Bai X, Chen X et al (2013) Construction of a transgenic pig model overexpressing polycystic kidney disease 2 (PKD2) gene. *Transgenic Res* 22:861–867
- Hingorani SR, Wang L, Multani AS, Combs C, Deramaudt TB, Hruban RH et al (2005) Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell* 7:469–483
- Hoffman EP, Brown RH Jr, Kunkel LM (1987) Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell* 51:919–928
- Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S et al (1996) Correlative memory deficits, A β elevation, and amyloid plaques in transgenic mice. *Science* 274:99–102
- Hsieh-Li HM, Chang JG, Jong YJ, Wu MH, Wang NM, Tsai CH et al (2000) A mouse model for spinal muscular atrophy. *Nat Genet* 24:66–70
- Humphries MM, Rancourt D, Farrar GJ, Kenna P, Hazel M, Bush RA et al (1997) Retinopathy induced in mice by targeted disruption of the rhodopsin gene. *Nat Genet* 15:216–219
- Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammer RE, Herz J (1993) Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *J Clin Invest* 92:883–893
- Jacobsen JC, Bawden CS, Rudiger SR, McLaughlan CJ, Reid SJ, Waldvogel HJ et al (2010) An ovine transgenic Huntington's disease model. *Hum Mol Genet* 19:1873–1882
- Jiang C, Ting AT, Seed B (1998) PPAR- γ agonists inhibit production of monocyte inflammatory cytokines. *Nature* 391:82–86
- Kashiwakura Y, Mimuro J, Onishi A, Iwamoto M, Madoiwa S, Fuchimoto D et al (2012) Porcine model of hemophilia A. *PLoS One* 7(11):e49450. doi:10.1371/journal.pone.0049450
- Kimberling WJ, Kumar S, Gabow PA, Kenyon JB, Connolly CJ, Somlo S (1993) Autosomal dominant polycystic kidney disease: localization of the second gene to chromosome 4q13–q23. *Genomics* 18:467–472
- Klymiuk N, Blutke A, Graf A, Krause S, Burkhardt K, Wuensch A et al (2013) Dystrophin-deficient pigs provide new insights into the hierarchy of physiological derangements of dystrophic muscle. *Hum Mol Genet* 22:4368–4382
- Knowles JW, Maeda N (2000) Genetic modifiers of atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 20:2336–2345
- Koenig M, Beggs AH, Moyer M, Scherpf S, Heindrich K, Bettecken T et al (1989) The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. *Am J Hum Genet* 45:498–506
- Kragh PM, Nielsen AL, Li J, Du Y, Lin L, Schmidt M et al (2009) Hemizygous minipigs produced by random gene insertion and handmade cloning express the Alzheimer's disease-causing dominant mutation APPsw. *Transgenic Res* 18:545–558
- Lanoix J, D'Agati V, Szabolcs M, Trudel M (1996) Dysregulation of cellular proliferation and apoptosis mediates human autosomal dominant polycystic kidney disease (ADPKD). *Oncogene* 13:1153–1160
- Lauer N, Suvorova T, Rütger U, Jacob R, Meyer W, Harrison DG et al (2005) Critical involvement of hydrogen peroxide in exercise-induced up-regulation of endothelial NO synthase. *Cardiovasc Res* 65:254–262
- Leblond V, Aufran B, Cesbron JY (1997) The SCID mouse mutant: definition and potential use as a model for immune and hematological disorders. *Hematol Cell Ther* 39:213–221
- Leonard WJ (1996) The molecular basis of X-linked severe combined immunodeficiency: defective cytokine receptor signaling. *Annu Rev Med* 47:229–239
- Leuchs S, Saalfrank A, Merkl C, Flisikowska T, Edlinger M, Durkovic M et al (2012) Inactivation and inducible oncogenic mutation of p53 in gene targeted pigs. *PLoS One* 7:e43323. doi:10.1371/journal.pone.0043323
- Li XJ, Li S (2012) Influence of species differences on the neuropathology of transgenic Huntington's disease animal models. *J Genet Genomics* 39:239–245
- Li T, Snyder WK, Olsson JE, Dryja TP (1996) Transgenic mice carrying the dominant rhodopsin mutation P347S: evidence for defective vectorial transport of rhodopsin to the outer segments. *Proc Natl Acad Sci U S A* 93:14176–14181
- Lorson CL, Hahnen E, Androphy EJ, Wirth B (1999) A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. *Proc Natl Acad Sci U S A* 96:6307–6311
- Lorson MA, Spate LD, Samuel MS, Murphy CN, Lorson CL, Prather RS et al (2011) Disruption of the Survival Motor Neuron (SMN) gene in pigs using ssDNA. *Transgenic Res* 20:1293–1304
- Lu W, Peissel B, Babakhanlou H, Pavlova A, Geng L, Fan X et al (1997) Perinatal lethality with kidney and pancreas defects in mice with a targeted Pkd1 mutation. *Nat Genet* 17:179–181
- Lu H, Huang D, Ransohoff RM, Zhou L (2011) Acute skeletal muscle injury: CCL2 expression by both monocytes and injured muscle is required for repair. *FASEB J* 25:3344–3355
- Luo Y, Li J, Liu Y, Lin L, Du Y, Li S et al (2011) High efficiency of BRCA1 knockout using rAAV-mediated gene targeting: developing a pig model for breast cancer. *Transgenic Res* 20:975–988
- Mannucci PM, Tuddenham EG (2001) The hemophilias—from royal genes to gene therapy. *N Engl J Med* 344:1773–1779
- Martignoni M, Groothuis GM, de Kanter R (2006) Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opin Drug Metab Toxicol* 2:875–894
- Mashimo T, Takizawa A, Voigt B, Yoshimi K, Hiai H, Kuramoto T et al (2010) Generation of knockout rats with X-linked severe combined immunodeficiency (X-SCID) using zinc-finger nucleases. *PLoS One* 5:e8870. doi:10.1371/journal.pone.0008870
- Mashimo T, Takizawa A, Kobayashi J, Kunihiro Y, Yoshimi K, Ishida S et al (2012) Generation and characterization of severe combined immunodeficiency rats. *Cell Rep* 2:685–694
- McCalla-Martin AC, Chen X, Linder KE, Estrada JL, Piedrahita JA (2010) Varying phenotypes in swine versus murine transgenic models constitutively expressing the same human Sonic hedgehog transcriptional activator, K5-HGLI2 Delta N. *Transgenic Res* 19:869–887
- McCreath KJ, Howcroft J, Campbell KH, Colman A, Schnieke AE, Kind AJ (2000) Production of gene-targeted sheep by nuclear transfer from cultured somatic cells. *Nature* 405:1066–1069
- Midgley CA, Lane DP (1997) p53 protein stability in tumour cells is not determined by mutation but is dependent on Mdm2 binding. *Oncogene* 15:1179–1189
- Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, Saris JJ et al (1996) PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* 272:1339–1342
- Monani UR, Lorson CL, Parsons DW, Prior TW, Androphy EJ, Burghes AH et al (1999) A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. *Hum Mol Genet* 8:1177–1183

- Morrison AE, Ludlam CA, Kessler C (1993) Use of porcine factor VIII in the treatment of patients with acquired hemophilia. *Blood* 81:1513–1520
- Mousavi SA, Berge KE, Leren TP (2009) The unique role of proprotein convertase subtilisin/kexin 9 in cholesterol homeostasis. *J Intern Med* 266:507–519
- Mussolino C, Cathomen T (2013) RNA guides genome engineering. *Nat Biotechnol* 31:208–209
- Nakamura A, Takeda S (2011) Mammalian models of Duchenne Muscular Dystrophy: pathological characteristics and therapeutic applications. *J Biomed Biotechnol* 2011:184393. doi:10.1155/2011/184393
- Nauck MA, El-Ouaghli A, Gabrys B, Hücking K, Holst JJ, Deacon CF et al (2004) Secretion of incretin hormones (GIP and GLP-1) and incretin effect after oral glucose in first-degree relatives of patients with type 2 diabetes. *Regul Pept* 122:209–217
- Nissen SE, Wolski K (2007) Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 356:2457–2471
- Noguchi M, Yi H, Rosenblatt HM, Filipovich AH, Adelstein S, Modi WS et al (2008) Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. *J Immunol* 181:5817–5827
- Olsson JE, Gordon JW, Pawlyk BS, Roof D, Hayes A, Molday RS et al (1992) Transgenic mice with a rhodopsin mutation (Pro23His): a mouse model of autosomal dominant retinitis pigmentosa. *Neuron* 9:815–830
- Parekh AK, Barton MB (2010) The challenge of multiple comorbidity for the US health care system. *JAMA* 303:1303–1304
- Petters RM, Alexander CA, Wells KD, Collins EB, Sommer JR, Blanton MR et al (1997) Genetically engineered large animal model for studying cone photoreceptor survival and degeneration in retinitis pigmentosa. *Nat Biotechnol* 15:965–970
- Pignatelli PM, Pound SE, Carothers AD, Macnicol AM, Allan PL, Watson ML et al (1992) Multipoint mapping of adult onset polycystic kidney disease (PKD1) on chromosome 16. *J Med Genet* 29:638–641
- Plum L, Wunderlich FT, Baudler S, Krone W, Brüning JC (2005) Transgenic and knockout mice in diabetes research: novel insights into pathophysiology, limitations, and perspectives. *Physiology (Bethesda)* 20:152–161
- Plump AS, Smith JD, Hayek T, Aalto-Setälä K, Walsh A, Verstuyft JG et al (1999) Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* 71:343–353
- Pritchard L, Sloane-Stanley JA, Sharpe JA, Aspinwall R, Lu W, Buckle V et al (2000) A human PKD1 transgene generates functional polycystin-1 in mice and is associated with a cystic phenotype. *Hum Mol Genet* 9:2617–2627
- Renner S, Fehlings C, Herbach N, Hofmann A, von Waldhausen DC, Kessler B et al (2010) Glucose intolerance and reduced proliferation of pancreatic beta-cells in transgenic pigs with impaired glucose-dependent insulinotropic polypeptide function. *Diabetes* 59:1228–1238
- Renner S, Römisch-Margl W, Prehn C, Krebs S, Adamski J, Göke B et al (2012) Changing metabolic signatures of amino acids and lipids during the prediabetic period in a pig model with impaired incretin function and reduced beta-cell mass. *Diabetes* 61:2166–2175
- Renner S, Braun-Reichhart C, Blutke A, Herbach N, Emrich D, Streckel E et al (2013) Permanent neonatal diabetes in INS(C94Y) transgenic pigs. *Diabetes* 62(5):1505–1511
- Reznikov LR, Dong Q, Chen JH, Moninger TO, Park JM, Zhang Y et al (2013) CFTR-deficient pigs display peripheral nervous system defects at birth. *Proc Natl Acad Sci U S A* 110:3083–3038
- Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK (1998) The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 391:79–82
- Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z et al (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245:1066–1073
- Rogers CS, Hao Y, Rokhlina T, Samuel M, Stoltz DA, Li Y et al (2008a) Production of CFTR-null and CFTR-DeltaF508 heterozygous pigs by adeno-associated virus-mediated gene targeting and somatic cell nuclear transfer. *J Clin Invest* 118:1571–1577
- Rogers CS, Stoltz DA, Meyerholz DK, Ostedgaard LS, Rokhlina T, Taft PJ et al (2008b) Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. *Science* 321:1837–1841
- Ross JW, Fernandez de Castro JP, Zhao J, Samuel M, Walters E, Rios C et al (2012) Generation of an inbred miniature pig model of retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 53:501–507
- Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N et al (1996) Secreted amyloid beta-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* 2:864–870
- Schnieke AE, Kind AJ, Ritchie WA, Mycock K, Scott AR, Ritchie M et al (1997) Human factor IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts. *Science* 278:2130–2133
- Schrank B, Götz R, Gunnarsen JM, Ure JM, Toyka KV, Smith AG et al (1997) Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. *Proc Natl Acad Sci U S A* 94:9920–9925
- Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W et al (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A* 110:3507–3512
- Sommer JR, Estrada JL, Collins EB, Bedell M, Alexander CA, Yang Z et al (2011) Production of ELOVL4 transgenic pigs: a large animal model for Stargardt-like macular degeneration. *Br J Ophthalmol* 95:1749–1754
- Soutar AK (2011) Unexpected roles for PCSK9 in lipid metabolism. *Curr Opin Lipidol* 22:192–196
- Spurney CF (2011) Cardiomyopathy of Duchenne muscular dystrophy: current understanding and future directions. *Muscle Nerve* 44:8–19
- Stoltz DA, Rokhlina T, Ernst SE, Pezzulo AA, Ostedgaard LS, Karp PH et al (2013) Intestinal CFTR expression alleviates meconium ileus in cystic fibrosis pigs. *J Clin Invest* 123:2685–2693
- Suzuki S, Iwamoto M, Saito Y, Fuchimoto D, Sembon S, Suzuki M et al (2012) Il2rg gene-targeted severe combined immunodeficiency pigs. *Cell Stem Cell* 10:753–758
- Takakura A, Contrino L, Zhou X, Bonventre JV, Sun Y, Humphreys BD et al (2009) Renal injury is a third hit promoting rapid development of adult polycystic kidney disease. *Hum Mol Genet* 18:2523–2531
- Takeuchi A, Irizarry MC, Duff K, Saito TC, Hsiao Ashe K, Hasegawa M et al (2000) Age-related amyloid beta deposition in transgenic mice overexpressing both Alzheimer mutant presenilin 1 and amyloid beta precursor protein Swedish mutant is not associated with global neuronal loss. *Am J Pathol* 157:331–339
- Tan W, Carlson DF, Lancto CA, Garbe JR, Webster DA, Hackett PB et al (2013) Efficient nonmeiotic allele introgression in livestock using custom endonucleases. *Proc Natl Acad Sci U S A* 110:16526–16531
- Thomas S, Kotamraju S, Zielonka J, Harder DR, Kalyanaraman B (2007) Hydrogen peroxide induces nitric oxide and proteasome activity in endothelial cells: a bell-shaped signaling response. *Free Radic Biol Med* 42:1049–1061
- Toschi V (2010) OBI-1, porcine recombinant Factor VIII for the potential treatment of patients with congenital hemophilia A and alloantibodies against human Factor VIII. *Curr Opin Mol Ther* 12:617–625
- Uchida M, Shimatsu Y, Onoe K, Matsuyama N, Niki R, Ikeda JE et al (2001) Production of transgenic miniature pigs by pronuclear microinjection. *Transgenic Res* 10:577–582
- Umeyama K, Watanabe M, Saito H, Kurome M, Tohi S, Matsunari H et al (2009) Dominant-negative mutant hepatocyte nuclear factor 1alpha

- induces diabetes in transgenic-cloned pigs. *Transgenic Res* 18:697–706
- Vickers P (2009) Severe combined immune deficiency: early hospitalisation and isolation. Wiley-Blackwell, Chichester
- Wei J, Ouyang H, Wang Y, Pang D, Cong NX, Wang T et al (2012) Characterization of a hypertriglyceridemic transgenic miniature pig model expressing human apolipoprotein CIII. *FEBS J* 279:91–99
- Whyte JJ, Samuel M, Mahan E, Padilla J, Simmons GH, Arce-Esquivel AA et al (2011) Vascular endothelium-specific overexpression of human catalase in cloned pigs. *Transgenic Res* 20:989–1001
- Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M et al (1996) Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). *Nature* 384:455–458
- Yamagata K, Yang Q, Yamamoto K, Iwahashi H, Miyagawa J, Okita K et al (1998) Mutation P291fsinsC in the transcription factor hepatocyte nuclear factor-1alpha is dominant negative. *Diabetes* 47:1231–1235
- Yamakawa H, Nagai T, Harasawa R, Yamagami T, Takahashi J, Ishikawa KI et al (1999) Production of transgenic pig carrying MMTV/v-Haras. *J Reprod Dev* 45:111–118
- Yang SH, Cheng PH, Banta H, Piotrowska-Nitsche K, Yang JJ, Cheng EC et al (2008) Towards a transgenic model of Huntington's disease in a non-human primate. *Nature* 453:921–924
- Yang D, Wang CE, Zhao B, Li W, Ouyang Z, Liu Z et al (2010) Expression of Huntington's disease protein results in apoptotic neurons in the brains of cloned transgenic pigs. *Hum Mol Genet* 19:3983–3994
- Yang D, Yang H, Li W, Zhao B, Ouyang Z, Liu Z et al (2011) Generation of PPARgamma mono-allelic knockout pigs via zinc-finger nucleases and nuclear transfer cloning. *Cell Res* 21:979–982
- Ye J, He J, Li Q, Feng Y, Bai X, Chen X et al (2013) Generation of c-Myc transgenic pigs for autosomal dominant polycystic kidney disease. *Transgenic Res*, Mar 30 [Epub ahead of print]
- Zaidi A, Schmoeckel M, Bhatti F, Waterworth P, Tolan M, Cozzi E et al (1998) Life-supporting pig-to-primate renal xenotransplantation using genetically modified donors. *Transplantation* 65:1584–1590
- Zhang SH, Reddick RL, Piedrahita JA, Maeda N (1992) Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science* 258:468–471
- Zuccato C, Valenza M, Cattaneo E (2010) Molecular mechanisms and potential therapeutical targets in Huntington's disease. *Physiol Rev* 90:905–981