Genetically engineered animal models have enabled unprecedented advancement in biomedical research. Many organisms have been used to produce genetically engineered models, such as mice, rats, sheep, cattle and pigs. All of these species have provided information that is valuable for the understanding of basic biology as well as the pathogenesis of many human diseases. However, some models have limitations in their ability to recapitulate a human disease or syndrome. For example, mice and rats are not the optimal animal model for studying the human eye or performing orthopedic surgery as a result of the limitations of the size of their eye or bones and joints, respectively. In addition, classical rodent models do not always fully recapitulate human diseases, such as cystic fibrosis.

The pig has many advantages over other species as a biomedical model and is believed to be the optimal model for xenotransplantation, risk assessment of environmental contaminants and drug discovery. In many respects, the pig is more similar to the human than other models in terms of anatomy, physiology and pathophysiology; phylogenetically, pigs are threefold closer to humans on the nucleotide level than are mice. We currently have a vast amount of information about the pig from its use in agriculture that touches on all aspects of the pig’s anatomy and physiology, addressing the gamut from basic genetic responses to the environment to applied aspects such as housing. In addition, we are able to apply many of the advances in medical technologies that have been developed for humans to the pig model, such as magnetic resonance imaging and positron emission tomography scans, and surgical training has been routinely performed in the pig. In this review, we will discuss the value of the pig as a biomedical model, as well the genomic tools and services, such as the NSRRC, that are available to facilitate its use in research pertaining to human health and diseases.

The pig as an experimental model
Animal models have long been used to replicate human diseases or conditions in attempts to develop better treatments and therapies. However, there are many factors that need to be considered when choosing an animal model to recapitulate specific symptoms. Does the animal model live long enough to develop the condition? Is the animal model easy to handle and does it fit into the animal facility? What is the growth rate of the animal model? Does the model provide enough measurable sample points during disease development? Classically, rodents have been the model of choice because they are easy to handle, require little space to maintain and can be bred to produce large numbers in a relatively short time frame. However, rodents have many anatomical and physiological differences with humans that can limit their applicability, and there are a number of rodent models that do not fully recapitulate the human disease, such as mouse models of amyotrophic lateral sclerosis, Alzheimer’s disease, CF, cancer models and Parkinson’s disease. These limitations leave investigators in search of a good animal model for their human disease or condition. In the past several years, the use of the pig as a biomedical model has increased markedly, largely as a result of increasing recognition of the similarities between the pig and human.

In its ‘natural’ state, the pig has been used in research in the areas of toxicology, exercise physiology, obesity and regenerative medicine. A recently published correspondence suggested that, in 56% of cases, the pig was the large animal of choice for regenerative
Cystic fibrosis

CF is an autosomal recessive disorder caused by a mutation in the CFTR gene, which regulates anion transport. In approximately 70% of humans with CF, the mutation in CFTR is a deletion of three base pairs that code for amino acid 508 (phenylalanine) of the CFTR protein. Some of the symptoms of CF include mucus blockage of the intestines (meconium ileus), blocked pancreatic ducts, congealed gallbladder and lung disease. When the CFTR gene is mutated in the mouse model, the animal’s channel function is altered, but there are no observed classical CF symptoms. However, when the model animal is a pig with the human mutation (that is, deletion of phenylalanine 508), 100% of the piglets exhibit classic CF symptoms, including meconium ileus, liver lesions and lung disease. The current pig model is being used to invasively investigate lung disease for the development of new therapies.

Creating genetically engineered pigs

Pig models have a predominant role in studies of retinitis pigmentosa (RP). RP is a human autosomal dominant disorder in which a common form of the disease arises from a substitution of a histidine for the amino acid proline at the 23rd position (P23H) in the RHO gene. RP is characterized by the onset of night blindness, loss of peripheral vision and then loss of central vision. In 2012, Ross et al. developed an inbred miniature pig model for RP that replicated the human phenotype. A human transgene with the P23H mutation in the human RHO gene was randomly inserted into the pig genome and a variety of phenotypes were recovered. Although there are several other RP models that have recapitulated the disease, the advantage of the pig model is the biological similarity between the pig and human eye. Another advantage of this pig model is that the inbred miniature pig genetic background facilitates the development of cell-based therapies. Researchers can inject large volumes of inoculum into the pig eye, allowing them to study the effects of cell-based therapies without any confounding effects of rejection. This model has been used for several experiments, such as determining whether the dietary supplement curcumin can arrest or delay rod photoreceptor degeneration.

Cancer

Cancer is vast collection of diseases that are typically characterized by abnormal cell growth. Mutations in KRAS account for about a quarter of human cancers and mutations in p53 account for another third. We have developed an inducible porcine cancer model with a floxed stop codon between the chicken beta actin promoter and mutant forms of KRAS and p53. This swine cancer model allows tumors to be induced in any tissue or organ with administration of CRE recombinase in the desired tissue. CRE promotes recombination between the two loxp sites and removes the stop codon. The removal of the stop codon results in transcription and subsequent translation of the two cancer inducing genes. Initial in vitro work with cells isolated from founder animals revealed that the transgene was functional when an adenovirus was used to deliver Cre to the cells in vitro. Cell morphology, division time and cell migration time were different in the cancer model cell line compared with the control lines. In addition, 12 of 14 mice injected with cells from the adeno-Cre-induced pig cancer model formed measurable tumors. Finally tumors were induced with adeno-Cre in three different locations in the KRAS and p53 pig.

Creating genetically engineered pigs

The technologies for producing genetic modifications to the pig genome have advanced tremendously over the past three decades. Historically, genetic engineering in pigs was accomplished with the addition of transgenes into random locations in the genome. Using a microinjection technique, genomic constructs were inserted into the pronucleus of zygotes, resulting in random insertional events with the potential to cause insertional mutations. The insertions can occur as single or multiple copies of the transgene integrating at one or more locations in the genome. In addition to the randomness of insertion, some of the injected transgenes may not integrate until after the zygote cleaves, that is, during the two-cell stage. Integration during the two-cell stage can occur at different locations (or not at all) in the genome in the two blastomeres, resulting in a mosaic embryo and animal. If the descendants of the two blastomeres contributed differentially to the germline then the transgene may or may not be transmitted to offspring. But even with these caveats, transgenesis by pronuclear injection is still a powerful tool to produce a new protein in the pig.

The next major advance in genetic engineering came as a result of the development of techniques for somatic cell nuclear transfer (SCNT). Given that somatic cells can be genetically engineered and used as donor cells for SCNT, those somatic cells that have the desired modification events can be propagated, genotyped and, depending on the model, examined for in vitro expression before SCNT and embryo transfer. The newly created cells can then be used to create founder animals with the predicted
Focus on Reproducibility

Table 1 | A partial list of the various swine genetic modifications available through the NSRRC

| Stock number | Strain name       | Background | Affected gene(s) | Genomic alteration                           |
|--------------|-------------------|------------|------------------|----------------------------------------------|
| NSRRC:0001   | Truline Hampshire | Outbred    | N/A              | Wild type                                    |
| NSRRC:0002   | Truline Duroc     | Outbred    | N/A              | Wild type                                    |
| NSRRC:0003   | Truline Landrace  | Outbred    | N/A              | Wild type                                    |
| NSRRC:0004   | Truline Large White | Outbred    | N/A              | Wild type                                    |
| NSRRC:0005   | Minnesota Mini    | Closed population | N/A        | Wild type                                    |
| NSRRC:0008   | Ossabaw           | Closed population | N/A          | Wild type                                    |
| NSRRC:0009   | GGTA1 knockout; CD55 | Closed population | CD55 and GGTA1 | GGTA1 knockout and CD55 transgene            |
| NSRRC:0012   | Yucatan           | Inbred     | N/A              | Wild type                                    |
| NSRRC:0013   | NIH Mini g/g      | Inbred     | N/A              | Wild type                                    |
| NSRRC:0014   | NIH Mini c/c      | Inbred     | N/A              | Wild type                                    |
| NSRRC:0015   | NIH Mini a/a      | Inbred     | N/A              | Wild type                                    |
| NSRRC:0016   | GFP NT92          | Outbred    | GFP              | Transgene: CAG promoter driving enhanced green fluorescent protein |
| NSRRC:0017   | Rhodopsin         | NIH/c/c    | RHO              | Transgene: rhodopsin (RHO) P23H substitution |
| NSRRC:0019   | FIX               | Outbred    | FIX, FURIN, VWF  | Transgenes: human coagulation factor IX; human alpha 1-antitrypsin; von Willibrand factor |
| NSRRC:0033   | Onco-pig          | Minnesota Mini | KRAS512D and p53R172H | Transgene: CAG laxP stop driving KRAS512D and p53R172H |
| NSRRC:0035   | RAG2 KO           | Minnesota Mini | RAG2      | RAG2 knockout                                 |
| NSRRC:0061   | CD39 and CD55     | Minnesota Mini | GGTA1    | GGTA1 knockout with human decay-accelerating factor (CD55) and CD39 transgenes inserted into GGTA1 |

For a complete list of all 61 strains available and for more details about the NSRRC, please refer to the website, http://www.nsrrc.missouri.edu.

The most recent and important advance in the genetic engineering of pigs is the use of gene-editing technologies such as zinc-finger nucleases29–30, transcription-activator-like effector nucleases31–33 and the clustered regularly interspaced short palindromic repeats/Cas9 system (CRISPRs/Cas9)34. These tools are highly efficient and can be designed to not leave a genetic footprint, such as a selectable marker. In fact, the CRISPR/Cas9 system is so efficient that injection into zygotes (if the individual CRISPRs are prescreened) can result in all of the offspring carrying edited alleles34. The CRISPR/Cas9 system has allowed us to edit the pig genome by knocking out genes and targeting insertion of donor DNA; it even has the potential to edit a single base in the genome. Because of the new gene-editing techniques, it is possible to envision creating virtually any modification or combination of modifications in pigs, thereby eliminating any remaining genetic barriers that have limited their use in the past.

NSRRC

Given that pigs are not as widely used compared with rodents, investigators seldom have the expertise or appropriate facilities to house and care for these animals, nor do they have the expertise to create the desired genetic modifications to address their disease of interest. The US National Institutes of Health (NIH) therefore created the NSRRC in 2003 to develop the infrastructure to ensure that biomedical investigators across a variety of disciplines have access to critically needed swine models of human health and disease. The NSRRC has several functions: importation of existing swine genetic models for human health and diseases; cryopreservation of all existing swine models for human health and disease; re-derivation/elimination of pathogens to improve the quality of the animal models for research; herd health monitoring for distribution of animals; distribution of pigs, cells, tissues and organs; creation of custom-generated swine models for human health and disease; cryopreservation of all existing models; and research. Table 1 lists some of the wild-type and genetically modified models available from the NSRRC for distribution to not-for-profit institutions. For a complete list of all 61 strains available and for more details about the NSRRC, please refer to the website, http://www.nsrrc.missouri.edu.
sensory perspective, I propose a different approach that focuses on reproducibility. This approach involves carefully controlling variables and ensuring consistency in experimental design and data analysis. By adopting this perspective, researchers can enhance the reliability and reproducibility of their findings, facilitating better collaboration and more accurate scientific progress. In conclusion, the focus on reproducibility is crucial for advancing scientific knowledge and ensuring the credibility of research results.
of the requested models. The NSRRC typically produces three to five models per year.

Cryopreservation
Germplasm from all genetically modified and wild-type animals is cryopreserved in different formats that include embryos, somatic cells and spermatozoa. Cryopreservation provides the NSRRC the ability to maintain lines without continuous production as well as to prevent catastrophic loss from disease outbreaks. To ensure maintenance of the lines, duplicate copies of the samples are stored off-site in a secondary storage facility. Cryopreserved samples can be shipped to the requesting investigator to create the animals on site at their facility. In addition, the NSRRC can provide an investigator with cryopreservation and storage services for their genetically modified swine model; those who do not wish to donate their models can still receive services, but on a fee-for-service basis.

Research and fee-for-service research
The NSRRC conducts research that will benefit its productivity in terms of performing its functions. The NSRRC conducts research in the areas of genetic engineering, somatic cell nuclear transfer, cryopreservation and health monitoring assay development. In addition, the NSRRC will aid investigators with their biomedical research projects. The NSRRC staff and requesting investigator will discuss the project to determine whether it is feasible for the NSRRC to conduct the research at their facility. Highly demanding projects and those requiring highly specialized equipment may be more difficult for the NSRRC to complete and may need to be conducted at the requesting PI’s facility. The investigator and the NSRRC will also discuss the budget and time frame needed to complete the project.

Conclusions
There has been a noticeable increase in the use of swine as models for human health and diseases over the past decade. Whether used in a natural state or genetically engineered, swine models are becoming the biomedical model of choice, largely as a result of their increasing ability to recapitulate various human diseases. With limited expertise and facilities across the country, the need for a central resource center has become a priority. The NSRRC was established to serve as a repository for valuable swine models for biomedical research, shifting the burden for maintaining and distributing swine models from the investigator to a national resource center. In addition, the NSRRC creates and distributes PI-driven custom-generated swine models. We are here to help the swine research community in laying the foundation for their biomedical research.

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