When will dual-purpose pigs fly?

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Domestic pigs (*Sus scrofa domesticus*) have a rich history, intertwined with diverse religious and cultural contexts, as well as intriguing biological characteristics. Since Neolithic times, these medium-sized mammals have been domesticated from wild boars in various regions, such as East Asia and Europe. For thousands of years, they have been primarily raised for meat purpose and have been featured prominently in many popular cuisines. From meals on the table to miniature pets, from religious ceremonies to scientific research, pigs hold a significant role across various facets of human society. The development of novel gene editing technologies and advancements in somatic cell nuclear transfer have significantly enhanced the efficiency of genetically modified pig generation, such as endogenous gene knockout pigs, precise single base substitution pigs, and exogenous gene knockin pigs. These pigs may possess specific traits that are considered highly promising for applications in both agriculture and biomedicine (see Figure 1).

AGRICULTURAL APPLICATION

Chinese native pig breeds commonly exhibit noticeable disadvantages, including slow growth, low lean-meat content, and inefficient feed conversion. In traditional pig breeding, there are practice limitations, such as long breeding cycles and high costs, highlighting the urgent need for adopting innovative modern approaches to revitalize the pig breeding industry.

To meet the increasing demands for animal protein worldwide, applications of the CRISPR-Cas9 technology hold promise in transforming the field by precisely modifying specific genes associated with desired traits in pigs. For instance, to improve production performance of the Guangdong small-ear spotted pig, single-cell colonies were generated with biallelic mutations in the myostatin (*MSTN*) gene and insulin-like growth factor 2 (*IGF2*) intron-3 locus using efficient CRISPR-Cas9-mediated gene editing. The system was also employed to target *CD163* and *CD1D* genes, and offspring piglets with such modifications exhibited resistance to porcine reproductive and respiratory syndrome virus (PRRSV).

Some favorable traits of livestock arise from multiple single-nucleotide polymorphisms at the same locus. Compared with the CRISPR-Cas9 genome editing technique, a newly developed single base editing technique targeting genomic loci of multiple genes, shows superior accuracy and biosafety in the genetic modification of pigs, without inducing double-strand breaks. Through direct zygote microinjection of the cytidine base editor system, genetically engineered pigs displayed improved growth performance and enhanced resistance to infectious diseases through mutation in *CD163* and *MSTN* genes and increased expression of *IGF2*.[1] Certainly, the genetic editing of animals raises social and

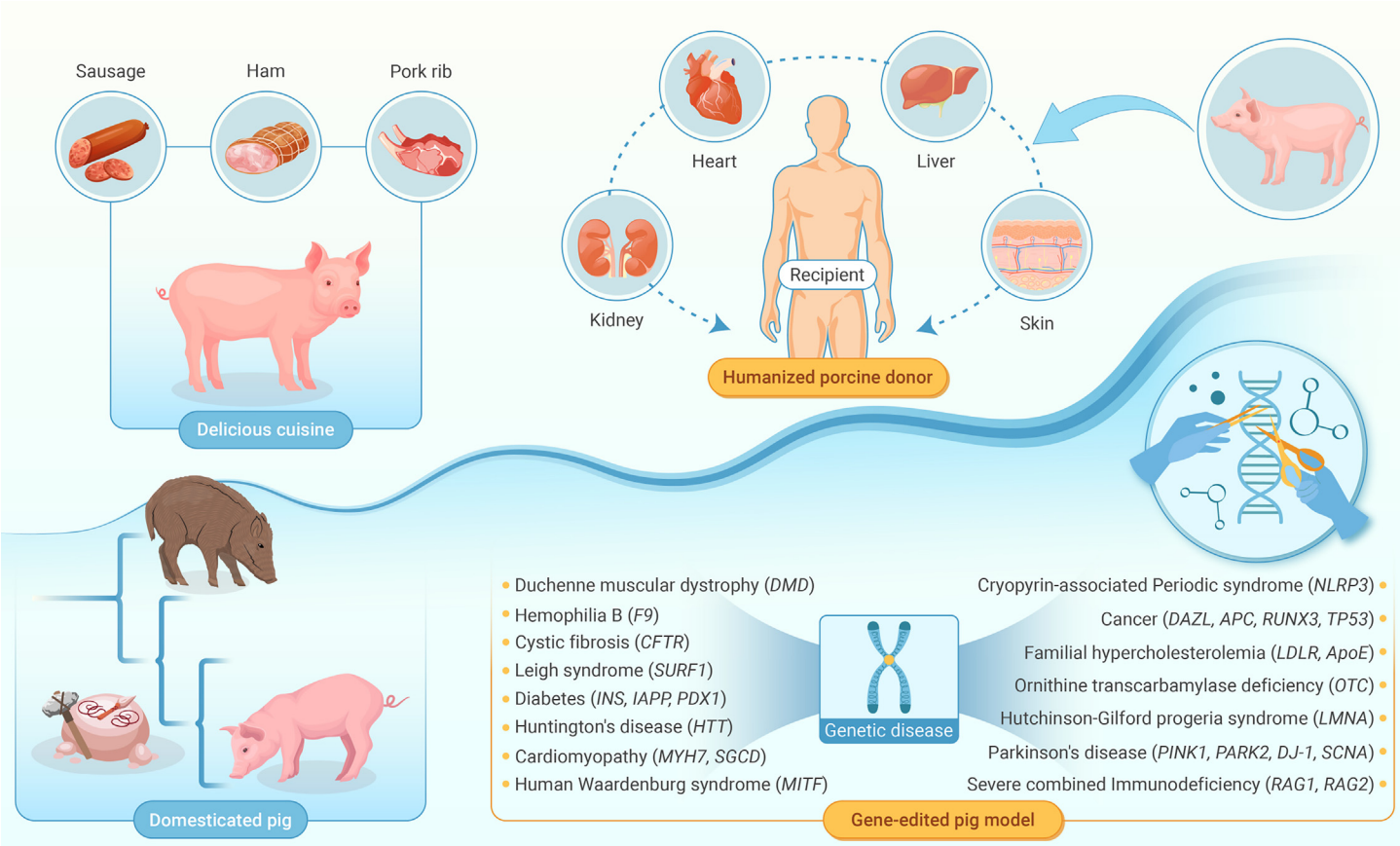

Figure 1. Schematic diagram of the utilization of pigs for agricultural and biomedical purposes The history of pig utilization is closely connected to agriculture and biomedicine. Throughout history, pigs have primarily been raised for meat purposes, featured in various popular cuisines worldwide. Advancements in gene editing technologies have facilitated the rapid development of genetically modified pigs. For biomedical use, by introducing targeted genetic modifications, researchers can create pig models that accurately mimic human diseases, thereby enhancing the understanding of disease mechanisms and the development of novel therapies. Nowadays, genetically modified pigs are being explored as potential organ donors for xenotransplantation.

ethical issues, including concerns related to animal welfare, ecological impact, food safety, ethical considerations, and regulatory policies. Comprehensive measures are essential to minimize potential animal suffering during gene editing, enforce stringent environmental monitoring to prevent ecological imbalance, ensure the safety of food products from genetically edited animals, maintain ethical standards to prevent misuse of the technology, and establish robust legal and regulatory frameworks to govern its development and application.

In 2020, GalSafe pigs were approved by the US Food and Drug Administration for human food consumption to reduce severe allergic reactions to galactose-α-1,3-galactose on the surface of porcine cells. The approval provided significant support for the application of gene editing technology in agriculture and food production, thereby unlocking the potential of gene editing in agricultural use. Meanwhile, galactose-removed pigs are also utilized in biomedical applications (discussed in the next section). In terms of environmental protection, a transgenic pig that expresses phytase in its salivary glands has been successfully generated, offering a unique biological approach to address both phosphorus nutrition and potential manure pollution in the pork industry.

Through integrating whole-genome selection technology with comprehensive phenotypic data collection during breeding processes, the field of pig breeding will undergo a revolutionary transformation in the future. Although gene editing has made significant strides, certain limitations still need to be addressed. We recommend focusing on discovering more functional genes to expand the pool of swine breeding materials, particularly those related to feed efficiency, carcass quality, reproductive performance, and environmental adaptability.

Currently the scale of high-quality omics remains insufficient, particularly due to a lack of core algorithms. Through multi-omics association analysis and pooled library screening for genome-wide knockout and CRISPRa/i, scientists anticipate gaining a deeper understanding of the interrelationships among various genes, which will enhance their ability to create pigs with an optimal balance of multiple desired traits. Moreover, multiple gene edits in pigs have been combined to decrease simultaneous susceptibility to PRRSV, transmissible gastroenteritis virus, and porcine deltacoronavirus.[2] The additive, dominant, and epistatic effects of genes on quantitative traits are significant and cannot be overlooked.

BIOMEDICAL APPLICATION

The close physiological similarities between pigs and humans make genetically engineered pig lines a potentially valuable resource for biomedical research, holding immense promise for groundbreaking discoveries through their being utilized as disease models and organ donors.

Given that pigs are more similar to humans than rodents in terms of physiology, drug metabolism, and disease progression, pig models, including genetically edited pigs, play a critical role in facilitating the development and clinical translation of complex treatment and therapeutics, such as those for cystic fibrosis (*CFTR*), severe combined immunodeficiency (*RAG1*, *RAG2*), cancer (*DAZL*, *APC*, *RUNX3*, *TP53*), cardiomyopathy (*MYH7*, *SGCD*), cryopyrin-associated periodic syndrome (*NLRP3*), diabetes (*INS*, *IAPP*, *PDX1*), Duchenne muscular dystrophy (*DMD*), familial hypercholesterolemia (*LDLR*, *ApoE*), human Waardenburg syndrome (*MITF*), hemophilia B (*F9*), Huntington's disease (*HTT*), Hutchinson-Gilford progeria syndrome (*LMNA*), Leigh syndrome (*SURF1*), ornithine transcarbamylase deficiency (*OTC*), Parkinson's disease (*PINK1*, *PARK2*, *DJ-1*, *SCNA*), and COVID-19 (*ACE2*).[3,4]

The demand for transplants exceeds the availability of organ donors. The concerns regarding interspecies immune rejection, transmission of porcine endogenous retrovirus (PERVs), and blood coagulation dysfunction have been alarming, but there are encouraging signs of improvement. To address the current shortage of organ donors, the clinical application of porcine xenogeneic organs might be a potential and attractive solution for recipients. Although PERVs have been experimentally shown to infect some human cells *in vitro*, there have been no documented cases of infection during xenotransplantation. Regarding regenerative medicine, technologies such as tissue engineering, bioprinting, and organoids offer promising alternatives to animal models. Though they provide tools to study human diseases and develop personalized medical therapies, these technologies are still in the quite early stages of development and face challenges in replicating the complexity and functionality of whole organs.

The survival time of genetically modified porcine organs in various recipients, including baboon, humans who suffered brain death, and deceased humans, has been continuously extended. At the University of Maryland Medicine, a 57-year-old patient, who received the world's first porcine-to-human heart transplantation, died from heart failure 2 months after the surgery.[5] On March 16, 2024, a 62-year-old man with end-stage kidney disease became the first patient to receive a kidney from a genetically modified pig. The transplanted kidney demonstrated the capability to produce urine, remove waste products from the blood, maintain body fluid balance, and perform other essential functions. However, the patient passed away approximately 2 months after the procedure. Subsequently, a 54-year-old woman became the second living individual to receive a transplant of a gene-edited pig kidney at New York University Langone Health. Unfortunately, on the 47th day post-surgery, the transplanted kidney had to be removed due to insufficient blood flow, and the patient died on the 86th day. In a recent study, a human-pig chimeric middle-stage kidney was generated in engineered *SIX1/SALL1*-null pig embryos through embryo complementation with pluripotent stem cells, which opened an exciting avenue for regenerative medicine. On May 17, 2024, a 71-year-old male patient with giant right lobe liver cancer became the first living person to receive a liver xenotransplantation from a genetically modified pig at the First Affiliated Hospital of Anhui Medical University, China. As reported by *Nature News*, the patient showed no hyperacute or acute rejection reactions within 7 days after surgery, and his coagulation system was not impaired either.

These pioneering cross-species transplant cases offer comfort and hope to severely ill patients who lack suitable human organs. Despite numerous benefits, fundamental work remains to be done before safe and effective xenotransplantation becomes a full-scale clinical trial. It is hard to answer when such trials involving pig organs will become a reality. Based on existing data, humans receiving porcine organs have a relatively shorter survival time compared to primate recipients. One way to improve the success rates and overall efficacy of the procedure is to explore highly effective immunosuppressive regimens and optimize genetic modification combinations by identifying additional genes that can trigger rejection reactions *in vivo*. It is worth noting that humans and primates, such as *Macaca fascicularis*, differ at the genetic level. Integrating *in vivo* animal experiments, surgeries on brain-dead human recipients, and genomics comparative analysis across species *in silico* could help scientists learn more beyond limited human clinical trials. Moreover, host genetics and environmental factors, including those related to the intestinal microbiome, jointly determine the phenotype of an organism. Genetically modified donor pigs typically live in highly hygienic environments, such as defined pathogen-free or specific-pathogen-free conditions. However, this controlled environment may increase the susceptibility of porcine organs to diseases or allergies after transplantation, especially when exposed to environments with unknown bacterial communities. In-depth investigation of immune incompatibility through alterations in the gut microbiome holds promise for personalized and better-tolerated therapies.

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DECLARATION OF INTERESTS

The authors declare no competing interests.