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## Review

## Viewing the current situation of pig model application in China's medical field from the application and funding of NSFC

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## ABSTRACT

**Background:** The National Natural Science Foundation of China (NSFC) is an important part of China's innovation system. In the last decade, the pig has become more and more widely used in the field of medical science, especially in otology research.

**Objective:** By analyzing and summarizing the funding information over recent years, we intend to identify the characteristics and trends of funding for research using pig models and provide references for future development.

**Material and methods:** This is a comprehensive analysis of features in funding for research projects involving pig models by the NSFC in the past 10 years, with a focus on projects in the field of otolaryngology/head and neck surgery.

**Results:** Both the number and amount of funding provided by the NSFC for research involving pig models are on the rise with each passing year. Researchers at the PLA General Hospital have completed a number of studies using miniature pigs in cochlear morphology, electrophysiology, cochlear implantation, cochlear transcription analysis, gene therapy, inner ear disease modeling and Eustachian tube pathology modeling.

**Conclusion:** Pigs as an ideal large mammal model are well suited in the current national basic research strategy in China, and can help further strengthen China's leading position in basic research in the world.

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## 1. Introduction

The National Natural Science Foundation of China (NSFC) is the main channel for funding basic research in China, an important platform for cultivating talents for scientific and technological innovation, an important guarantee for balanced and coordinated disciplinary development, the source of innovation-driven development strategies, and an important bridge for international scientific and technological cooperation and exchanges. Applications for funding by the NSFC represent the consensus and research level of various science branches and reflect the understanding of the front-line researchers on the subject and the trend of personal research interest. The NSFC has become an important part of China's national innovation system. A review of information on projects supported by the NSFC over recent years will contribute to understanding NSFC's roles in the development of scientific disciplines, to characterizing current trends of disciplinary development, and to providing useful indicators for future development.

Animal models are important experimental tools in biomedical research, which can help us understand the pattern of disease occurrence and progression and find prophylactic-therapeutic measures. Among medical laboratory animals, pigs are a species most closely related to humans outside primates. Because of their high degree similarity with humans in anatomy, genetics and pathophysiology, they have drawn widespread attention for their important position in the field of medical science. In otology research, common animal models traditionally used are rodents, whose inner ear is small in size and late in maturity with different anatomy compared to humans. These differences further limit application of rodents as surgical models in clinical otology research. In comparison, pigs and humans share highly similar auditory organs in morphology and structure, especially in middle ear cavity and pharyngeal lymph tissue, making them very suitable for modeling otitis media (Pracy et al., 1998). The thickness and strength of cochlear wall in pigs are similar to that in humans, and pig cochleae have been widely used in surgical training for cochlear fenestration and in high resolution cochlear CT scanning (Sepehr et al., 2008). At birth, pig inner ear is basically mature with normal hearing, just like humans. Therefore, application of pig models has great potentials in the field of otology (Yang, 2016). This paper provides a comprehensive review of projects using pig models supported by the NSFC in recent years, with a focus on application of pig models in the field of otolaryngology.

## 2. Materials and methods

The Big Data Knowledge Management Service Portal of NSFC (Fig. 1) was searched to retrieve information on funded projects. The collected information included: application code (Department of Medical Sciences), program category (18 items in total), project keywords ("pig model") and year of approval (2010–2019). The retrieved information was recorded in Excel for statistical analysis and mapping.

## 3. Results

### 3.1. Funded medical science projects involving pig models by discipline

From 2010 to 2019, 97 medical science projects funded by the NSFC contained the keyword "pig", including 49 General Program projects, 22 Young Scientists Fund projects, 13 Fund for Less Developed Regions projects, 10 Emergency Management Project of the Department of Management Science projects, 2 International

(Regional) Cooperation and Exchange Programs projects, and 1 Programs of Joint Funds project (Table 1). The majority of funded projects were General Program projects (51%), followed by Youth Science Fund projects (23%) and Fund for Less Developed Regions projects (13%), with the three making up 87% of all funded projects, similar to the pattern of funding for all disciplines. The amount of funding was directly proportional to the number of funded projects, reaching 10.73 million yuan in 2019, the highest in the decade (Fig. 1). Both the number and amount of funding showed a trend of steady increase, with a small spike in 2011–2012. In 2019 (Zhang, 2019), with the overall rate of funding at the lowest, the number and amount of funded pig model related projects reached the highest point in history (Fig. 2).

### 3.2. Distribution of funded projects among disciplines

The distribution of NSFC funded projects among disciplines can reflect the level and application degree in the field of pig model. During the 2010–2019 period, the first level application codes of the top eight disciplines in medical science (accounting for 65% of total applications) that applied for projects involving pig models were H18 (medical imaging and biomedical engineering,  $n = 12$ ), H02 (circulatory system,  $n = 10$ ), H07 (endocrine system, metabolism and nutrition,  $n = 8$ ), H14 (oral craniomaxillofacial science,  $n = 8$ ), H13 (otolaryngology head and neck science,  $n = 7$ ), H06 (locomotor system,  $n = 7$ ), H03 (digestive system,  $n = 6$ ), and H15 (emergency and critical care medicine/trauma/burns/plastic surgery,  $n = 5$ ) (see Fig. 3 for details).

### 3.3. Funding of key programs

Projects funded by the NSFC Key Program, Major Program, Major Research Plan, International (Regional) Cooperation Research & Exchange Programs and Special Fund for Research on National Major Research Instruments often reflect the country's major strategic needs and important explorations at scientific frontiers in recent years, and represent scientific innovation abilities and breakthroughs at the highest level in China. Among all the projects funded by the NSFC Department of Health Sciences during 2010–2019, two international (regional) cooperation and exchange projects were supported: i.e. the "establishment of a pig model of vestibular aqueduct syndrome deafness and gene therapy research" project by Yang Shiming, the PLA General Hospital, in 2018 (funding amount: 2.4 million yuan, project No.: 81820108009, application code: H1305 - otorhinolaryngologic genetics and development related diseases), and the "China-Canada health research cooperation program: targeted research on treatment for antisense oligonucleotide mediated exon skipping in Duchenne muscular dystrophy using a transgenic pig model" project in 2013 by Yin Haifang of Tianjin Medical University (funding amount: 1 million yuan, project number: 81361128013, application Code: H0602 - locomotor system genetics).

### 3.4. Funding of projects involving pig models in the field of otolaryngology head and neck science

From 2010 to 2019, seven projects in the field of otolaryngology, head and neck science related to pig models were funded, accounting for 7% of the total funded projects. See Table 2 for specific funded projects. From the distribution among host institutions in the table, the PLA General Hospital received most (57%) of the funding, showing an obvious dominance in this discipline, followed by the PLA Third Military Medical University (29%) and Nanjing Medical University.

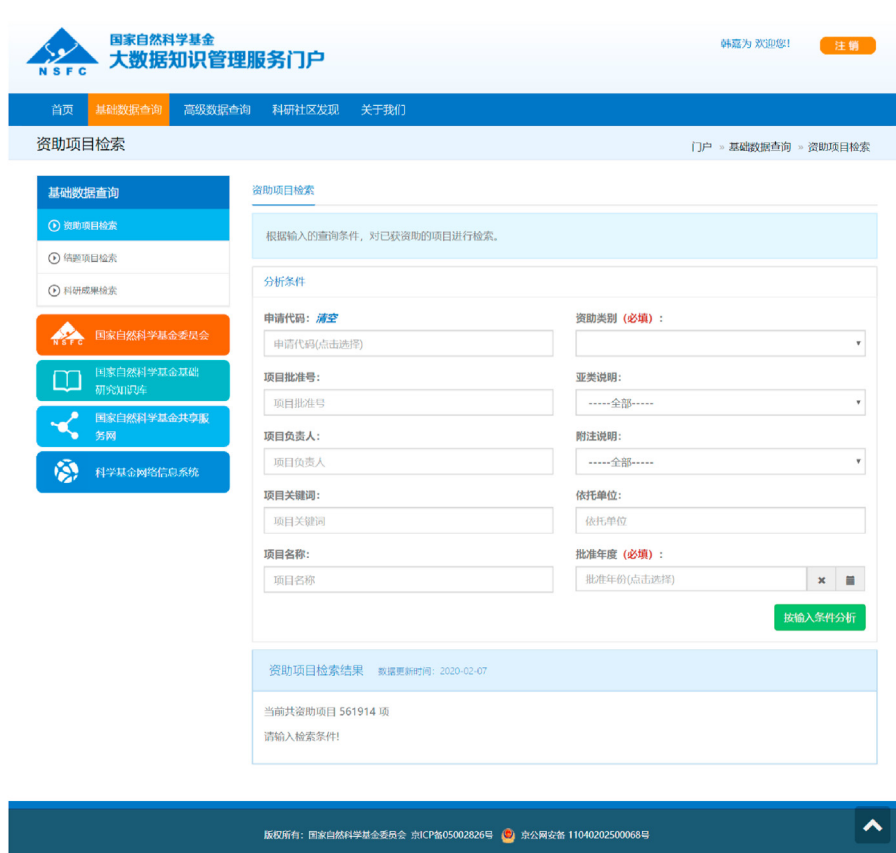


Fig. 1. Big Data Knowledge Management Service Portal of NSFC interface.

**Table 1**  
Projects involving pig models funded by the NSFC during 2010–2019.

	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2010–2019
General Program	2	8	7	3	2	2	6	9	4	6	49
Young Scientists Fund	1	1	2	2	3	2	3	4	4		22
Fund for Less Developed Regions		1	1	1	2	2		1	3	2	13
Emergency Management Project of the Department of Management Science		1		1			1			7	10
Programs of Joint Funds				1							1
International (Regional) Cooperation and Exchange Programs									1		2
Total Number	3	11	11	8	7	6	10	14	12	15	97
Total Amount (million yuan)	0.86	6.07	6.22	4.27	3.10	3.16	4.90	6.67	7.16	10.73	53.14

3.5. Academic achievements from NSFC funded projects involving pig models in the field of otology head and neck science

In regard to morphology of the pig cochlea, Guo et al. (2017) published an article on morphological and functional development of cochlear scala media lateral wall in miniature pigs. The study highlighted the development of stria vascularis in the embryonic stage, which matured in synchrony with the organ of Corti, with the endocochlear potential (EP) reaching a mature level on E98 day when the stria vascularis was formed. The work provided a morphological and functional basis for further studying the pathogenesis of various inner ear diseases using miniature pig models. Zhong et al. (2018) demonstrated three-dimensional structures of the vestibule and cochlea in miniature pigs using CT scanning and multi planar reconstruction (MPR) technologies. The results showed that parameters of the vestibule and cochlea in miniature pigs were consistent with those in humans, making miniature pigs a legitimate model for cochlear implantation studies. Yi et al. (2014)

completed measurement of the external ear, middle ear, inner ear and lateral recess in the miniature pig by microanatomy, showing similarities with those in human being and potential applicability of the miniature pig as an animal model for otology surgery studies.

In electrophysiology, Guo et al. (2015a) recorded ABRs, EP and K<sup>+</sup> concentration in the endolymph in 20 Chinese miniature pigs and studied hair cells morphology by electron microscopy, reporting morphological and electrophysiological characteristics of the Chinese miniature pig cochlea for the first time. Ma et al. (2016) found that presence of umbilical cord mesenchymal stem cells (UMSCs) in the cochlea might lead to change of ABR waveforms in pigs with congenital deafness and albinism.

For cochlear implant (CI), Chen et al. (2017) established a clinical animal model platform for cochlear implant research. A Rongchang pig model with normal hearing or hearing impairment was used to determine the success of implantation and optimal electrode positioning. Yi et al. (2016) evaluated and tested cochlear implants with EABRs and imaging in 10 animals. The results showed, in

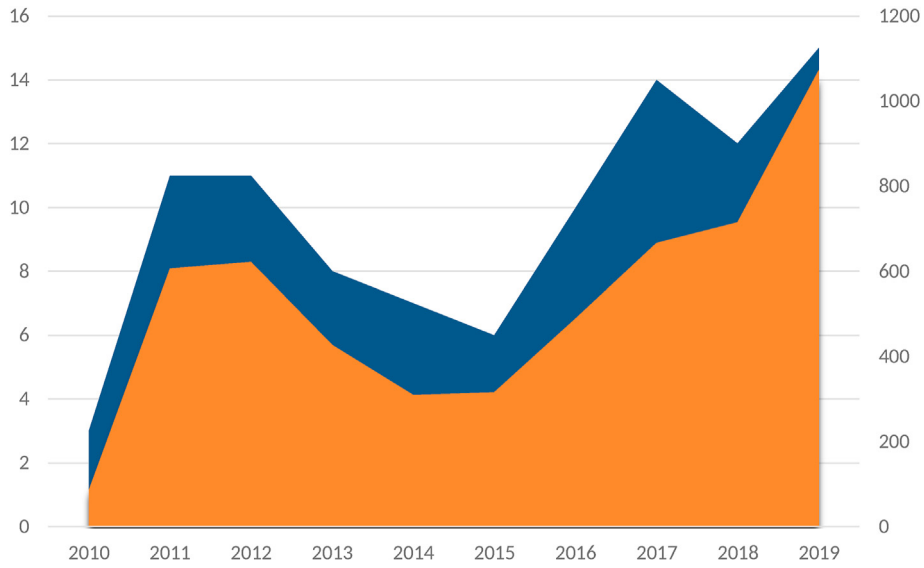


Fig. 2. Trend of funding for medical science projects involving pig models by the NSFC during 2010–2019.

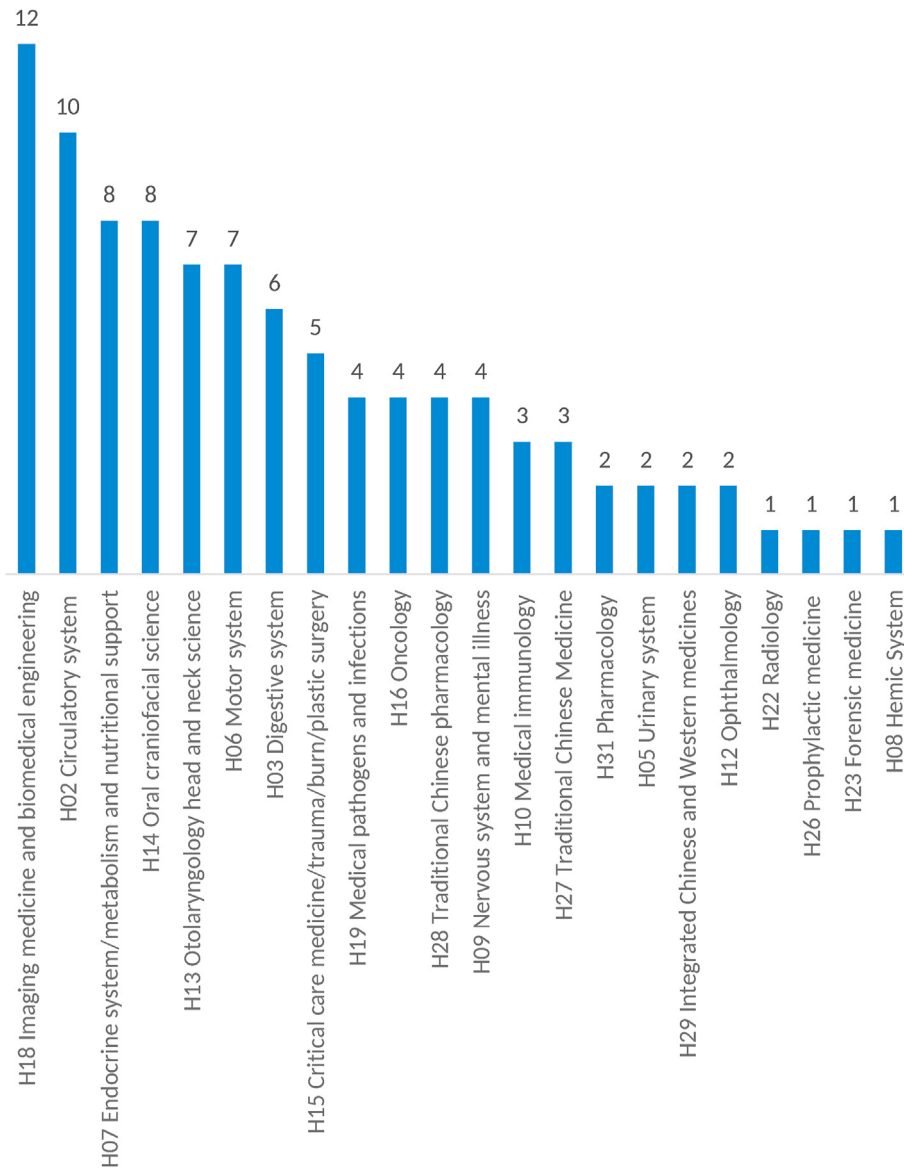


Fig. 3. Distribution of NSFC supported projects involving pig models across disciplines.

**Table 2**  
Programs involving pig models in otorhinolaryngology-head and neck science supported by the NSFC.

Project Title	Program Type	Project No.	Funding Amount (1,000 yuan)	P. I.	Host Institution	Year of Approval
Study on the mechanism of improving hearing function of WS deaf pigs induced by field potential of cochlear implant stem cell SGN enrichment and synergizing	General program	81970890	555.00	Wei Chen	Chinese PLA General Hospital	2019
Study on biological characteristics and molecular pathologic mechanism of congenital unilateral deafness pig model	General program	81970895	555.00	Hui Zhao	Chinese PLA General Hospital	2019
The establishment of a pig model of deafness with large vestibular aqueduct syndrome and the study of gene therapy	International (regional) cooperation and exchange program	81820108009	2,400.00	Shiming Yang	Chinese PLA General Hospital	2018
Establishment and application of a new miniature pig model of hereditary deafness with abnormal sterol metabolism	General program	81771000	800.00	Xin Cao	Nanjing Medical University	2017
Molecular mechanism of Mondini malformation in the inner ear based on a new model of hereditary deafness in pigs	General program	81670941	580.00	Ying Wang	Chinese PLA Third Military Medical University	2016
Establishment and characterization of a new model of syndromic hereditary deafness miniature pig with pigmentation disorders	General program	81570933	950.00	Haitao Shang	Chinese PLA Third Military Medical University	2015
Establishment of miniature pig animal model of human auditory neuropathy and its stem cell therapy	General program	81271082	850.00	Lidong zhao	Chinese PLA General Hospital	2012

comparison with traditional animal models, great similarity between the miniature pig and human, indicating its potential to become a useful model for CI research.

Through transcription analysis of cochlear development, Wang et al. (2017) found that the key period of genetic regulation of the embryonic cochlea in Bama miniature pigs was the E49–E56 days, at which time both genetic regulation and differentiation of the cochlea went through most rapid changes, while cellular characteristics of neural stem cells decreased most rapidly. This finding is of great significance for cochlea related research, especially regarding gene and stem cell therapies for deafness.

In studying gene therapy using miniature pigs, Shi et al. (2017) discussed expression of AAV1 in the miniature pig cochlea after transfection through the round window membrane, and found that the expression was mainly in the inner hair cells. In the same way, GFP expression began to appear at 2 weeks and peaked at 3 weeks, but was also found in Hensen's cells, pillar cells, outer pillar cells, the spiral limbus and spiral ligament. Ji et al. (2019) reported a surgical route of gene delivery in Bama miniature pigs that required minimal operation on the temporal bone.

The miniature pig model of MITF gene mutation can resemble Waardenburg syndrome. Chen et al. (2016) found that a mutation of MITF-M led to early degeneration of stria vascularis intermedia cells in the cochlea, as well as depigmentation, similar to the typical human Waardenburg syndrome phenotype. Guo (Guo et al., 2015b) et al. Studied transcription variation and expression patterns of the porcine MITF gene, and found a high degree homology between porcine and human MITF sequences. Chen et al. (2018) studied inner ear and cochlear histopathology in deaf pigs. They found a major defect in the stria vascularis in pigs with MITF variants, due to loss of neural crest derived melanocytes. They also proposed that MITF-M mutant pigs could provide an important animal model for cochlear implantation and cell therapy of patients with congenital hereditary deafness. In vestibular research, Du et al. (2019) studied characteristics and function of vestibular hair cells in miniature pigs with MITF-M mutation. The results showed that mutation of the MITF-M gene only affected saccular hair cells, but not other vestibular organs.

Hai et al. (2017a) established a pig model of Waardenburg syndrome type 2A (WS2A), and showed that heterozygous mutant pigs demonstrated obvious hearing loss and hypopigmentation in the skin, hair, iris and other parts of the body, accompanied by vascular degeneration, hair cell fusion and loss of EP, suggesting

that the pig model was very similar to the clinical symptoms and molecular pathology seen in human WS2A patients. This implies that the pig model can be applied to studying the etiology of human hearing loss and to developing new treatments.

Hai et al. (2017b) also confirmed the feasibility of artificial random mutation in pigs. In a successful and systematic three generation N-ethyl-N-nitrosourea (ENU) mutagenesis screening, a total of 6,770 G1 and 6,800 G3 pigs were screened, and 36 new pig pedigrees with 36 dominant and 91 recessive phenotypes were established. Further localization was carried out in 10 mutation families, and mutation of the SOX10 (R109W) gene was found to cause abnormal inner ear function similar to human Mondini malformation. Hao et al. (2018) studied the molecular mechanism of inner ear development in miniature pigs with Sox10 mutation and sensorineural deafness and found that Sox10 mutation resulted in incomplete partition of the cochlea, cystic apex, and cochlear defect and shortening. The model showed 173 differential expression genes (DEG) and 185 differential expression long-chain non coding RNA (lncRNA).

An et al. (2019) obtained qualitative and quantitative information on the eustachian tube (ET) by studying 16 domestic small experimental pigs and measuring their eustachian tube structures. Their anatomical study showed that the tympanum orifice and pharyngeal orifice were located at the anterior wall of the middle ear and the posterior end of the lateral wall of nose, respectively. The cartilaginous part runs through the entire ET, with its length as well as the diameter of the isthmus similar between humans and miniature pigs. Inclination of the ET in miniature pigs is greater than that in humans. There are slight differences in the general tissue structure between miniature pigs and humans, but the overall structure of ET in pigs and humans are similar, suggesting that miniature pig is a suitable ET model for clinical studies.

#### 4. Summary and outlook

As an ideal large mammal model, the pig has broad application prospects and an important position in basic research. Its high similarity with humans in anatomy and function ensures its unique advantages as an animal model for human diseases and pharmaceutical research, and as organ donors, as well as being useful in other areas (Yang, 2016). A comparative analysis of the topics and contents of projects involving pig models funded by the NSFC helps us grasp the key support areas and the direction of frontier issues

by the NSFC, while providing information on important achievements and contributions by pig models in the field of medical science. Judging by the number and amount of funded projects, it is clear that the NSFC has strengthened its support for research using pig models. From discipline distribution of NSFC funded projects, most pig models are used in research in the area of imaging medicine and biomedical engineering, circulatory system, locomotor system, oral craniomaxillofacial science, otorhinolaryngology head and neck science, digestive system, emergency and critical care/trauma/burns/plastic surgery. From supported key projects in the past three years, Otorhinolaryngology -head and neck science is a target area of state priority support. The PLA General Hospital team has taken a leading position in receiving to NSFC funding for otorhinolaryngology-head and neck science projects involving pig models.

In the past decade, China has been continuously increasing investment in projects, personnel, teams and research centers in the area of basic research, striving for scientific breakthroughs, as a fundamental strategy to ensure the future prosperity of the nation. 2020 is the end of the 13th five-year plan and the key year to look forward to the 14th five-year plan. Global competition and cooperation in science would take on a new pattern. Summarizing and analyzing NSFC funded projects in the past 10 years can help show future strategic development goals, and future priority funding fields and discipline development directions aimed to promote innovation breakthroughs by high-level talents and to further strengthen China's leading position in basic research in the world.

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