Use of large animal models to investigate Huntington's diseases

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Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder that is caused by a CAG repeat expansion in exon 1 of the HD gene. As a result, the CAG expansion encodes the polyglutamine (polyQ) repeat in the N-terminal region of the disease protein, huntingtin (Htt). The majority of HD patients carry expanded polyQ repeats in the range of 38–55 glutamines and develop progressive neurological symptoms that typically occur between the ages of 30 and 50 years. A large repeat longer than 60 glutamines can lead to juvenile-onset HD. The polyQ expansion causes the misfolding and aggregation of Htt and selective neurodegeneration that preferentially occurs in the striatum and extends to various brain regions as HD progresses. The late-onset and selective neurodegeneration and protein aggregation in HD are the common pathological features shared by other neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis. Because HD is caused by a single gene mutation, HD provides us with an ideal model to investigate how protein misfolding can cause selective neurodegeneration.

Identification of the genetic mutation in HD leads to generation of a variety of animal models that express expanded-polyQ containing Htt. By expressing mutant Htt containing an expanded CAG repeat in different species, a variety of genetically modified animal models of HD have been established and characterized. Among these models, mouse models of HD have been widely used and provided valuable information regarding the pathogenesis and therapeutic development of HD. These animal models offered clear evidence that small N-terminal Htt fragments carrying polyQ expansion are prone to misfolding and aggregation and also are more toxic than full-length mutant Htt, as transgenic Htt mouse models expressing small N-terminal Htt fragments die earlier and show more severe behavioral phenotypes than mice expressing full-length mutant Htt. Although these HD mouse models show age-dependent accumulation of mutant Htt and associated neurological symptoms, they lack overt and selective neurodegeneration, a typical pathological hallmark of HD. Similar to HD mouse models, other genetically modified mouse models, including those for AD and PD, express different types of misfolded proteins and also show the absence of selective neurodegeneration.
are thought to account for differential pathological changes in rodents and humans.12

Non-human primates have been used to generate transgenic monkey models to express disease genes or exogenous foreign genes.14–16 Of which, transgenic HD rhesus monkeys represent the first monkey model of human disease, which expresses exon1 mutant Htt with 84Q under the control of the human ubiquitin promoter.17 The HD monkeys were generated by injecting lentiviruses into fertilized oocytes to express mutant Htt. However, the HD monkey model shows much more severe phenotypes than the HD mouse model that was generated by the same strategy. Unlike transgenic mice, which can survive after birth when expressing the same exon1 mutant Htt with an even longer polyQ repeat (150Q),6 HD transgenic monkeys with 84Q could die postnatally.14 Despite their early death, transgenic monkeys developed key clinical HD features including dystonia, chorea, and seizure,14 which have not been replicated by mouse models and other small animal models. Also, the brains of HD monkeys show abundant Htt aggregates and axonal degeneration.14

The above findings suggest that large animals may be more sensitive to toxic Htt proteins than rodents. In support of this idea, transgenic pig model expressing the N-terminal mutant Htt (N208-105Q), which was generated via somatic cell nuclear transfer (SCNT), also showed the postnatal death phenotype and abundant Htt aggregates.17 In addition, the transgenic HD pig model displayed apoptotic cells and chorea, which were not found in transgenic HD mouse models.17

The significant differences between large and small transgenic HD animal models underscore the importance in using large animals to investigate HD neuropathology. These differences also point out that overexpression of N-terminal mutant Htt is very deleterious to large animals. Thus, it is important to establish a large animal model that expresses full-length mutant Htt at the endogenous level for investigating neuropathology and phenotypes.

Although non-human primates would be an ideal model to study HD because they are closer to humans than other animals, their long-breeding period and high cost as well as the difficulty in modifying endogenous monkey genes post considerable challenges to generate a monkey model that can endogenously express mutant Htt. On the other hand, pigs have several advantages over non-human primates for generating genetically modified animal models. The existing genetic manipulation tools enable the generation of a variety of pig models of human diseases.18,19 Somatic cell nuclear transfer (SCNT) in combination with CRISPR/Cas9 allows for genetic modifications of the endogenous pig genes.20–22 The SCNT leads to non-chimeric animals in the first generation that may recapitulate endogenous genetic mutation-associated phenotypes. In addition, the fast breeding period (5–6 months for sexual maturation) and large litter size (average 7–8 piglets) of pigs hold obvious advantages over non-human primates when considering the time line of generating large animal models of human diseases.

Recently, Yan and her colleagues used pigs to successfully establish the first large animal model that endogenously expresses full length mutant Htt.23 They applied CRISPR/Cas9 to insert a large CAG repeat (150 CAGs) into the endogenous pig Htt gene in cultured fibroblast pig cells and then used somatic nuclear transfer technology to generate HD knock in pigs that express full-length mutant Htt containing a 150 polyglutamine repeat.23 HD KI pigs show age-dependent neurological symptoms including body weight loss, early death, and movement difficulties. The brains of HD KI pigs also show the accumulation of Htt aggregates, similar to those in other HD animal models. More importantly, HD KI pig brains display the selective neurodegeneration in the striatum, recapitulating the important pathological feature of HD patients. Furthermore, the phenotypes and neurodegeneration are transmittable via germline, as F1 KI pigs show similar pathological phenotypes as the founder animals.23

Table 1 showed the differences and similarities between small and large animal models of HD.

Generation of a HD KI pig model demonstrates for the first time that large mammals can recapitulate overt and selective neurodegeneration and the severe symptoms caused by the mutant protein that is expressed at the endogenous level. The apparent differences in the pathology and phenotypes between small and large mammalian animal models of HD are likely attributable to the following facts. First, the species-dependent differences in lifespan, genomics, anatomy, and physiology play essential roles in determining the severity of neurodegeneration in different species. Indeed, the lack of distinguishable caudate nucleus and putamen structures in the rodent striatum accounts for the inability to mimic the preferential caudate degeneration in HD. Second, the development of the central nervous system is remarkably different in various species. The rapid development and maturation of the rodent brain may render neuronal cells resistant to toxic proteins. On the other hand, the toxic effect of misfolded proteins during the lengthy early brain development in large mammals may be required for the more severe neuropathology in adult brains after the differentiation and maturation of neuronal cells. Also, Htt in the brains of small and large mammals may associate with different partners and function differentially.

The examples of using large animals to investigate HD highlight the importance of the large animal models for investigating other neurodegenerative diseases. Although rodent models have

Table 1
Comparison of mouse and large animal models of HD.

| Transgene | Exon1 (1–67) Htt-150Q | N208-150Q | HD-KI pig |
|-----------|----------------------|-----------|-----------|
| Species   | Mouse                | Monkey    | Pig       |
| Lifespan  | Short life span 5–8 m| Embryonic or Postnatal death | Postnatal death |
| Atrophy   | Yes                  | Axonal degeneration | Apoptosis |
| Htt aggregates | Yes | Yes | Yes |

Fig. 1. Large animal models can be used for developing therapy.
Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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