The Libechov Minipig as a Large Animal Model for Preclinical Research in Huntington’s disease – Thoughts and Perspectives

Abstract
Large animal models to explore the safety and tolerability of novel therapeutic approaches for Huntington’s disease (HD) are in exploration to achieve higher translational reliability in future studies. Recently, a Libechov minipig has been established as one new transgenic (Tg) large animal model for HD. We here discuss the advantages and limitations in using this model in HD with regards to breeding, housing, handling, and with respect to homology to humans and ethical considerations. A group of TgHD and wild type (WT) female minipigs (n = 36) was used to gain first evidence about abovementioned aspects. It is concluded that Libechov minipigs may fulfill an important role to bridge the gap between rodents and non-human primates in the translation to humans.

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Key words
minipig – Huntington’s disease – animal model – phenotyping – magnetic resonance imaging – imaging – behavioral – preclinical research
Introduction

A wide range of transgenic (Tg) and knock-in animal models has been developed to explore the pathology, safety and efficacy of new therapeutic approaches for Huntington’s disease (HD) [1]. HD is an autosomal-dominant neurodegenerative disorder with motor, cognitive and behavioral symptoms [2,3]. It is caused by a CAG triplet repeat expansion ≥ 36 in the huntington gene that leads to neuronal dysfunction and death in wide areas of the brain including the cerebral cortex, white matter and striatum, due to the misfolded mutant huntingtin (mHTT) protein [4–8]. Established animal models are e.g. nematodes, drosophila, mice, rats, sheep, monkeys and minipigs [9,10]. So far especially the rodent models contributed a major part of the preclinical research in HD [11–13]. In spite of numerous preclinical findings, none of the compounds proposed for disease modifying treatments of HD based on preclinical data has been successfully translated into the clinic to date [14].

Large animal models have thus been proposed as a possible improvement for preclinical assessments with a higher probability for successful translation. One model recently established by the Research Center PIGMOD & Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Czech Republic, is the TgHD Libechov minipig [15]. We decided to explore the value of the Libechov minipig as an animal model for HD with respect to breeding, housing and handling, and particularly with regards to aspects such as the similarity to humans and ethical considerations (Fig. 1). The TgHD Libechov minipig exhibited a stable transmission of the HD mutation across several generations. The Libechov minipig was created by using lentiviral transduction. It expresses an N-terminal truncated form of human huntingtin with 124 CAG/CAA repeats on chromosome 1. We here present and discuss arguments for and against applying this minipig as a large animal model for HD based on experience gained with the Libechov minipigs in a long term follow-up study.

The Libechov minipigs

Animals and assessments

Tg and wild type (WT) Libechov minipigs (n = 36 total), bred in the Institute of Animal Physiology and Genetics of the Czech Academy of Science in Libechov, Czech Republic [15], were housed in Muenster. The animals arrived in the central animal facility of the University of Muenster, Germany, at an age of three months in six groups of six animals. The groups included female WT and TgHD animals. Each group was housed in a temperature and humidity controlled stable with a size of 2 m² per animal. The stables are enriched with toys, litter and hay. A daily veterinary care was provided and weight was monitored weekly. Before the battery of phenotyping assessments started the animals initially passed a short phase of anti-penic treatment. After successful habituation in the new environment the minipigs learnt to follow a target stick by using classical and operant conditioning to ensure a comfortable handling.

The battery of assessments developed and explored included several motor, cognitive and behavioral tests will be described elsewhere [16–20]. The prerequisite for performing this battery of tests was the feasibility to successfully handle the animals. Further the minipigs underwent magnetic resonance imaging (MRI) scans that included multiple anatomical, diffusion-weighted and spectroscopic sequences, which will also be described elsewhere in detail [21–24]. Precondition for performing MR Imaging during anesthesia was the feasibility to narcotize the animals for a longer period of time.

The experience with our 36 Libechov minipigs yielded a lot of arguments in favor of applying and further developing the minipig as a model for neurodegenerative disorders such as HD. However, we also discovered disadvantages of the model, which are discussed below.

Breeding compared to other animal models

Tab. 1 shows the generation times of mice, rats, sheep and pigs in view of sexual maturity, gestation period, litter size and length of estrus and estrous cycles [9,23,26]. With the sexual maturity of 5–8 months (pigs) and 3–10 months (sheep), and the gestation period of 114 days (pigs) and 150 days (sheep) large animals show a longer generation time than rodents. The litter size and the lengths of estrus and estrous cycles support the use of rodents when large numbers of animals and short timelines are required. While sheep lamb only two offspring, pigs are able to breed 9–12 piglets. Thus, litter size favors pigs over sheep. In general, bigger litter sizes of genetically changed animals enable faster and more economic breeding with fewer founder-animals.

![Fig. 1. Aim of this article.](image-url)

Discussion of the suitability of the Libechov minipig as an animal model for HD with respect to breeding, housing and handling, and particularly with regards to the similarity to humans and ethical considerations.
Lifespan and body weight compared to other animal models

Tab. 2 shows the lifespan of mice, rats, sheep and minipigs [27–33]. While rodents are short-lived, large animals have a lifespan up to 20 years. HD and other neurodegenerative disorders need many years to decades to manifest clinically. Therefore, a long lifespan may be advantageous to study the progression of disease with similar timelines than observed in human phenotype development.

Tab. 3 shows the average body weight of mice, rats, sheep, Goettingen and Libechov minipigs [9,26,34,35]. Female Libechov minipigs have a mean body weight of around 75 kg at an age of 30 months (body weight at the age of 30 months across 32 female Libechov minipigs); thus, the weight of these animals is comparable to adult humans. However, considerable variability between body weights can be observed in both the Libechov minipigs and humans. Nevertheless, preclinical research in this model permits pharmaceutical studies with a biodistribution pattern that should allow reliable translation to humans. Food intake of the Libechov minipig must be controlled and these animals have to be fed restrictively to avoid unpredictable weight gains. To optimize husbandry minipigs should be separated while fed, because they develop a hierarchy that results in unequal access to food with the strongest minipigs receiving most.

Brain volume and structure compared to other animal models

Tab. 4 and Fig. 2 show the brain volume and characteristics of the gross brain structure of mice, rats, minipigs, pigs, and sheep [9,36–39]. Brain volume and brain structure should be important aspects when selecting suitable animals for preclinical research in neurodegenerative disorders. Pigs, Libechov minipigs and sheep have a brain volume of 96–145 g, 90–100 g and 130–140 g, respectively. The brain size of human is approximately 1,300–1,400 g. Thus there still is a considerable difference between the brain size of large animals and humans. Nevertheless, the sheep’s, minipig’s and pig’s brain size and structure offer advantages compared to rodents as demonstrated by MRI and positron emission tomography (PET) applications in vivo. Large animals have a gyrencephalic brain similar to humans while rodents have a lissencephalic brain. The brain of minipigs is also similar to humans with regards to the blood supply and to immune response characteristics [32]. However, a serious disadvantage of minipigs compared to e.g. sheep are the large paranasal sinuses. This makes brain implants a difficult challenge in minipigs, while implants in sheep brains are possible due to a different anatomy [9].

Housing and handling of the Libechov minipigs

Six female groups with six WT and TgHD animals were housed in the central animal fa-
cility of the University of Muenster, Germany. Each group was housed in a stable with 2 m² per animal. The stables were temperature and humidity controlled. Pigs are not able to sweat. They increase their temperature by using other pigs or litter as heat source. To reduce temperature they lie alone and decrease their food intake. Adult pigs have a temperature optimum of 15–20 °C. In Muenster, all animals had the possibility to use toys like balls, chains and sisal 24 hours a day. Minipigs are curious animals and ready to explore new items. Balls and teeth rings were fixed with chains. Every month the toys were rotated between groups to preserve their interest. In addition, stables were enriched with chains, sisal, litter and hay (Fig. 3).

The minipigs exhibited high levels of motivation to cooperate with the experimenters. The animals were easy to handle and easily pleased for several years. However, work with minipigs requires more manpower and more space compared to work with rodents.

Female minipigs and castrated males live in groups. Social hierarchy between minipigs in one group is strong and persists. Because of this constant hierarchy behavioral changes with impact on social interaction should be readily detectable. Thus we expect that the complex social structure is another fea-

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**Tab. 4. Brain volume and brain structure compared between of mice, rats, sheep and pigs.**

<table>
<thead>
<tr>
<th>Brain weight and structure</th>
<th>mice (Mus musculus)</th>
<th>rats (Rattus norvegicus)</th>
<th>Goettingen minipig</th>
<th>Libechov minipig</th>
<th>merino sheep</th>
<th>pigs (Sus scrofa domestica)</th>
</tr>
</thead>
<tbody>
<tr>
<td>weight abs. (g)</td>
<td>0.3–0.4</td>
<td>2</td>
<td>80</td>
<td>90–100</td>
<td>130–140</td>
<td>96–145</td>
</tr>
<tr>
<td>gross brain structure</td>
<td>lissencephalic</td>
<td>lissencephalic</td>
<td>gyrencephalic</td>
<td>gyrencephalic</td>
<td>gyrencephalic</td>
<td>gyrencephalic</td>
</tr>
</tbody>
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Fig. 3. Enrichment: Two minipig-groups share a sisal toy (a).

A lot of different toys for pigs are available because of the well-established husbandry methods for the food industry; (b) Minipig-group in their 12m² stable enriched with litter and toys. Rest period after breakfast.
The feasibility to perform considerable advantage of large animal models may be disputed, the development of these models is work in progress. A considerable advantage of large animal models such as minipigs is the feasibility to perform assessments in vivo such as MRI, PET, CSF (Collecting Cerebrospinal Fluid), blood collection, and stereotactically-guided delivery of drugs into the brain [9,41]. Another advantage of large animals such as minipigs is the high genetic homology to humans, in general and with respect to the htt gene. The porcine htt gene, for instance, has higher genetic homology to humans (96%) [15] than the htt gene in rodents, e.g. mice with 91% [42]. However, the Libechov minipig currently used has limitations due to the genetic construct used, which only expresses a fragment of the N-terminal part of the huntington gene. In addition, the model uses a CAG/CAA repeat while the human huntingtin gene has a pure CAG repeat. The fact that only one transgene is inserted may be advantageous, however, it must be kept in mind that the huntingtin fragment is expressed with the background of two porcine huntington genes. Therefore a desirable next step would be the development of a humanized knock-in minipig model of HD.

Another serious limitation is the lack of reliable data on the time and course of phenotype development in the TgHD Libechov minipig model. However, characterization of the model with behavioral tests [16–20] and a range of imaging measures [21–24] translated from human studies such as TRACK-HD is ongoing and results will be available soon. Once the timeline and magnitude of phenotype measures is known, studies may expand from safety and tolerability to biomarker assessments, which are available today, to symptomatic and disease modifying trials using clinical endpoints.

**Conclusion**

In spite of the availability of several HD animal models, the pathomechanisms of HD are still not fully understood. Likely, the animal models established to date will not be able to answer relevant outstanding questions. All of them exhibit advantages and disadvantages (Fig. 5). Large animal models, such as the TgHD minipig discussed here, offer the unique opportunity to expand our knowledge. They may serve as a valuable compromise between scientific needs and environmental requirements. Thus they could occupy a central position between rodents and non-human primates, close the
gap between preclinical research in rodents and clinical research in humans and contribute to higher translational reliability and sensitivity in HD and beyond. The use of TgHD minipigs in preclinical studies is feasible, and a further development of this model both in terms of assessments and advances in genetic constructs is warranted.

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References