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Behavioral phenotyping of minipigs transgenic for the Huntington gene

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HIGHLIGHTS

- Large animal models such as minipigs provide new options for research in HD.
- Their long lifespan resembles human HD calling for sensitive phenotype assessments.
- Motor, cognitive and behavioral tests to assess the phenotype of minipigs are proposed.
- We demonstrate feasibility to perform these assessments in tgHD minipigs.
- The tests proposed facilitate observational and interventional trials in tgHD minipigs.

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ABSTRACT

Background: While several novel therapeutic approaches for HD are in development, resources to conduct clinical trials are limited. Large animal models have been proposed to improve assessment of safety, tolerability and especially to increase translational reliability of efficacy signals obtained in preclinical studies. They may thus help to select candidates for translation to human studies. We here introduce a battery of novel tests designed to assess the motor, cognitive and behavioral phenotype of a transgenic (tg) HD minipig model.
New methods: A group of tgHD and wildtype (wt) Libechov minipigs ($n = 36$) was available for assessment with (1) a gait test using the GAITRite® automated acquisition system, (2) a hurdle-test, (3) a tongue coordination test, (4) a color discrimination test, (5) a startbox back and forth test and (6) a dominance test. Performance of all tests and definition of measures obtained is presented.
Results: Minipigs were able to learn performance of all tests. All tests were safe, well tolerated and feasible. Exploratory between group comparisons showed no differences between groups of tgHD and wt minipigs assessed, but low variability within and between groups.
Comparison with existing method(s): So far there are no established or validated assessments to test minipigs in the domains described.
Conclusions: The data shows that the tests presented are safe, well tolerated and all measures defined can be assessed. Prospective longitudinal application of these tests is warranted to determine their test–retest reliability, sensitivity and validity in assessing motor, cognitive and behavioral features of tg and wt minipigs.

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1. Introduction

Huntington's Disease (HD) is characterized by a complex movement disorder including involuntary choreatic movements and deficits in motor coordination, cognitive and behavioral symptoms (Walker, 2007). These symptoms are explained by neuronal dysfunction and death in widespread gray and white matter areas of the brain with a particular focus in the basal ganglia (Aylward et al., 2013; Tabrizi et al., 2009; Tabrizi et al., 2013). The pathology is caused by an autosomal-dominant CAG triplet repeat expansion ≥36 in the Huntington gene (The Huntington's Disease Collaborative Research Group, 1993) that translates to a misfolded mutant Huntingtin (mHTT) protein.

The monogenetic background of HD facilitated the development of a wide range of transgenic (tg) and knock-in animal models that have been used to investigate HD pathology and assess safety and efficacy of novel treatments, including nematodes, drosophila, mice, rats, sheep, monkeys and minipigs (Morton and Howland, 2013; Pouladi et al., 2013). A large body of preclinical research has been conducted using rodent models and the need for these models has been undisputed (e.g., Crook and Housman, 2011; Kim et al., 2011; William and Gray, 2011), even if critically challenged (Philips et al., 2014). However, in spite of high standards that have evolved in the field (Menalled and Brunner, 2014), none of the compounds proposed for disease modifying treatments of HD based on preclinical data in previously established models has been successfully translated into the clinic (Venuto et al., 2012). With several promising novel treatment approaches on the horizon (Ross et al., 2014a; Wild and Tabrizi, 2014), but limited resources for conducting clinical trials in a rare disease (Reilmann, 2012; Sampaio et al., 2014), efforts to improve translational validation of treatment targets are warranted (Munoz-Sanjuan and Bates, 2011).

It was proposed that large animal models may have the potential to enhance translational reliability of preclinical research, e.g., by supplementing and confirming results obtained in rodents prior to translation into humans (Howland and Munoz-Sanjuan, 2014; Morton and Howland, 2013; Yang and Chan, 2011). Thus the development of large animal models for HD has recently been accelerated including the development of assessments for preclinical efficacy testing (Chang et al., 2015).

We here report the development of a novel behavioral assessment battery targeting the characterization of tgHD minipigs. Minipigs exhibit a high genetic homology with humans, have similar metabolism, body weight and a fairly large gyrated brain with more structural similarities to the human brain than most models routinely used up-to-date (Baxa et al., 2013). Minipigs are easy to breed and house and maintenance of colonies is feasible at lower costs compared to monkeys (Morton and Howland, 2013; Schramke et al., 2015). Minipigs were thus proposed as a model that could play an important role in certain areas of translational preclinical research including HD (Dolezalova et al., 2014; Vodicka et al., 2005). Recently, a transgenic minipig with stable transmission of the HD mutation across several generations was established (Baxa et al., 2013). The model was generated by lentiviral transduction using the Libechov minipig and expresses an N-terminal truncated form of human huntingtin with 124 CAG/CAA repeats on chromosome 1. Since minipigs exhibit a life expectancy of about 12 years, even the long repeat size of this model may require several years until a clinical phenotype is detectable (Schramke et al., 2015; Vodicka et al., 2005; Weiss et al., 2008). Therefore the development of sensitive assessments similar to human HD (Ross et al., 2014a)–see e.g., PREDICT-HD (Paulsen et al., 2014) and TRACK-HD (Tabrizi et al., 2013), is desirable to use the full potential of this model in a timely manner.

In human HD, several novel assessments including quantitative motor (Q-Motor) (Reilmann, 2012), cognitive and behavioral measures have been developed alongside MR imaging and demonstrated potential to serve as reliable measures or biomarkers of disease stage and progression (Paulsen et al., 2014; Tabrizi et al., 2009, 2012, 2013). Several of these approaches are currently explored in multicenter clinical trials (Sampaio et al., 2014). Some measures showed cross sectional and longitudinal changes in HD as early as in the second decade before predicted disease onset of clinical motor phenotype (Bechtel et al., 2010; Scahill et al., 2013; Stout et al., 2012; Tabrizi et al., 2009, 2012, 2013) as defined by current criteria (Reilmann et al., 2014).

We therefore decided to assess the feasibility to establish a battery of phenotypical tests targeting motor, cognitive and behavioral features in tgHD minipigs in a pre-defined, controlled setting. The assessments are aimed to enable sensitive and reproducible detection of phenotype and should inform the design of longitudinal observational studies and future preclinical therapeutic trials employing minipigs. A group of tgHD and wild-type Libechov minipigs underwent training and testing with the novel assessments introduced. We hypothesized that (1) conduct of these tests was feasible, and (2) the data collected were suitable to perform group comparison between tgHD and wild type (wt) Libechov minipigs sufficiently standardized to include them in longitudinal studies.

2. Materials and methods

2.1. Experimental animals

A group of female tgHD and wildtype (wt) Libechov minipigs ($n = 36$) of F1 to F3 generation were available for this study (Baxa et al., 2013). We used female animals because they are easier to handle and could be rather held in groups than male minipigs, if they were not castrated (Morton and Howland, 2013). They were bred in the Institute of Animal Physiology and Genetics in Libechov, Czech Republic. The Libechov minipig model is driven by a human HTT promoter carrying a truncated N-terminal fragment of huntingtin with 548 amino-acids containing a stable repeat with 124 CAG copies. Animals were received in six groups of six animals each between September 2012 and September 2013. Each group consisted of mixed wt and tgHD animals and was housed in a separate stable in the central animal facility at the University Hospital of Muenster, Germany. The stables with a size of 12 m^2 each were temperature and humidity controlled with a target value of 22° and 50–60% humidity Due to seasons minor variations could be measured. All groups were provided with litter and had access to different toys such as balls, chains and sisal. A continuous medical surveillance was provided. The minipigs regularly received parasite prophylaxis and hoof trimming. The weight was monitored weekly and added up to a range between 40 kg and 120 kg (adult female minipigs).

2.2. Experimental assessments and setup

All assessments were performed within the central animal facility. All study procedures were reviewed and approved by the local governmental animal protection agency prior to initiation of the study. After their arrival, animals initially received a short phase of "anti-panic treatment" to facilitate adjustment in the new environment and to calm down. The anti-panic treatment included the exploration of the new setup and habituation to the trainer and to the rewards. The minipigs learned to allow the touch of the trainer and to be alone in the startbox. They were then exposed to classical and operant conditioning procedures such as clicker-training and learning to follow a target stick.