Transgenic rat model of Huntington’s disease

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Huntington’s disease (HD) is a late manifesting neurodegenerative disorder in humans caused by an expansion of a CAG trinucleotide repeat of more than 39 units in a gene of unknown function. Several mouse models have been reported which show rapid progression of a phenotype leading to death within 3–5 months (transgenic models) resembling the rare juvenile course of HD (Westphal variant) or which do not present with any symptoms (knock-in mice). Owing to the small size of the brain, mice are not suitable for repetitive in vivo imaging studies. Also, rapid progression of the disease in the transgenic models limits their usefulness for neurotransplantation. We therefore generated a rat model transgenic of HD, which carries a truncated huntingtin cDNA fragment with 51 CAG repeats under control of the native rat huntingtin promoter. This is the first transgenic rat model of a neurodegenerative disorder of the brain. These rats exhibit adult-onset neurological phenotypes with reduced anxiety, cognitive impairments, and slowly progressive motor dysfunction as well as typical histopathological alterations in the form of neuronal nuclear inclusions in the brain. As in HD patients, in vivo imaging demonstrates striatal shrinkage in magnetic resonance images and a reduced brain glucose metabolism in high-resolution fluor-deoxy-glucose positron emission tomography studies. This model allows longitudinal in vivo imaging studies and is therefore ideally suited for the evaluation of novel therapeutic approaches such as neurotransplantation.

INTRODUCTION

Huntington’s disease (HD) is an autosomal dominant disorder caused by an expanded and unstable CAG trinucleotide repeat within the coding region of the HD gene (IT15)(1). The mutation leads to a progressive degeneration of neurons primarily in striatum and cerebral cortex. Clinically, HD is characterized by movement abnormalities, cognitive impairments, and emotional...
disturbances (2). In general, movement disturbances begin with chorea. Depressed mood and more subtle deficits apparent in neuropsychological tests may precede motor symptoms by years. The disease progresses relentlessly until death within 15–20 years. No effective treatment to influence the onset or the progression is presently available.

Many attempts have been made to generate animal models of HD. Excitotoxin models replicate many of the histological and neurochemical features as well as some of the motor and cognitive signs of HD (3–5), but neurodegeneration is not truly progressive. Therefore, their usefulness for the evaluation of treatment effects is limited.

Transgenic animal models of HD (6–11) provide new ways of studying the neuropathological mechanisms underlying HD. In particular the R6/2 transgenic mouse line, which expresses the first exon of the human HD gene carrying 141–157 CAG repeat expansions (6), develops a number of key features of HD, including progressive motor deterioration (12,13), appearance of neuronal intranuclear inclusions (14), discriminative learning impairments (15), and altered emotionality (16). However, R6/2 mice express very large numbers of CAG repeats that are only found in the juvenile type of HD. A rapid disease progression associated with diabetes in R6/2 mice (13) is not typical for the adult-type HD and may complicate the assessment of potential therapeutic approaches. Although HD transgenic mice provide important insights into the molecular basis of HD, there is still a need for animal models which resemble the common adult type of disease and which are more suitable for repetitive in vivo imaging. These rapidly emerging techniques offer the opportunity to compare directly the pathological alterations of the human condition with the corresponding animal model in longitudinal studies (17).

In this report, we describe the first transgenic rat model bearing a human HD mutation with a high-end adult onset allele of 51 CAG repeats that exhibits progressive neurological, neuropathological and neurochemical phenotypes closely resembling the common late manifesting and slowly progressing type of disease. We demonstrate that HD transgenic rats are well suited for complex behavioral studies and the evaluation of in vivo progression markers using high-resolution PET and MRI.

RESULTS

Generation of the HD transgenic rat model
A 1962 bp rat HD cDNA fragment (18) carrying expansions of 51 CAG repeats under the control of 885 bp of the endogenous rat HD promotor (19) was used for microinjection (Fig. 1A). Two founders were obtained and transgenic lines established. Of these, we followed up line 2762 for more than 2 years and found the CAG repeat length remaining stable in more than 147 meioses (data not shown). The mutant amino terminal portion of huntingtin is expressed in the brain as shown by western blot analysis (Fig. 1B), in particular in the frontal and temporal cortex, the hippocampus, the basal ganglia, and the mesencephalon, but at a much lower level in the cerebellum or the spinal cord (Fig. 1C).

![Figure 1](https://example.com/image1.png)

**Figure 1.** Transgene construct and huntingtin expression in transgenic rats. (A) The first 154 bp of a partial huntingtin cDNA spanning 1962 bp of the N-terminal rat sequence (RHD10) (18) were replaced by a PCR product from the affected allele of a HD patient. The cDNA is driven by a 885 bp fragment of the rat HD promotor (position −900 to −15 bp) (19). A 200 bp fragment containing the SV40 polyadenylation signal was finally added downstream of the cDNA resulting in RHD/Prom51A. (B) Western blot analysis of brain tissue of transgenic rat line 2771 and 2762 using polyclonal anti-huntingtin antibody 675 demonstrates a 75 kDa product representing the expression of the transgene although at a lower level than the endogenous protein. Homozygotic rats (+/+) express about double the amount of the transgene protein as hemizygous lines (+/−). (C) Western blot analysis of tissue from different brain areas of transgenic rat line 2762 at the age of 6 months, demonstrating a 75 kDa product representing the expression of the transgene in the frontal cortex, the temporal cortex, hippocampus, basal ganglia and mesencephalon, but not in the cerebellum or the spinal cord. However, overexposure of the same western blot clearly demonstrates that the transgene is also expressed in the cerebellum and the spinal cord though at a much lower level (data not shown).
Slow progressive phenotypes with emotional, cognitive and motor dysfunction

At birth we found transgenic rats and wild-type littermates phenotypically indistinguishable. Transgenic rats of both sexes are fertile without any sign of atrophy of the sexual organs. We observed a lower body weight gain in transgenic rats that was slowly progressive with the animals being about 20% lighter at the age of 24 months (Fig. 2A). At this age, transgenic rats commonly died after a 2 week period of rapid weight loss, which is associated with emaciation and muscular atrophy (Fig. 2B). Plasma glucose levels were always normal in routine screening (data not shown).

Transgenic animals often showed opisthotonus-like movements of the head. No resting tremor, ataxia, clumping, vocalizations, dyskinesia or seizures were observed. Except for occasional dyskinetic movements of the head, overt behavioral abnormalities were only found on dedicated behavioral testing.

At the age of 2 months transgenic rats developed a reduction of anxiety-like behavior in the elevated plus maze test (Fig. 2C), which is similar to the findings in R6/2 transgenic mice (16). At the age of 10 months transgenic rats showed cognitive decline in a spatial learning task for testing working memory in the radial maze (Fig. 2D and E). At the age of 5 months we had no indication of motor dysfunction in the animals (Fig. 2F), while at the age of 10 and 15 months progressive impairments of hind- and forelimb coordination and balance in the accelerated test were found (Fig. 2G and H). Thus, as in HD patients, emotional and cognitive impairments preceded progressive motor deterioration.

Accumulation of huntingtin aggregates and nuclear inclusions in striatal neurons

We examined whether mutant huntingtin forms aggregates and inclusions in the brain of 18-month-old rats using EM48, a rabbit antibody selective for mutant huntingtin (20,21). Most of the EM48 immunoreactive products appeared as punctuate labeling in the striatum, especially in the ventral region near the lateral ventricles and in the caudal part (Fig. 3B). Occasionally EM48 labeled aggregates were observed in the cortex. Other regions including hippocampus and cerebellum showed very weak or no EM48 label. In wild-type animals no EM48 labeled aggregates or puncta were found (Fig. 3A).

Two types of EM48 labeling, neuropil aggregates and nuclear inclusions were observed. As in other HD animal models (11,22) and in HD patient brains (20) some neuropil aggregates were arranged in linear arrays and most of them were scattered (Fig. 3C). Single nuclear inclusions were mainly observed in the striatum (Fig. 3D), resembling other HD mouse models (14,21). Since the striatal projection neurons terminate their axons in the lateral globus pallidus (LGP), we also examined the caudal region of the striatum. Nuclear staining and neuropil aggregates were common in the striatum. In the LGP, however, most EM48 labeling existed as neuropil aggregates.

To examine at what age mutant huntingtin forms aggregates and inclusions in the ventral region of the striatum, we additionally screened brains of 1-, 6-, 12- and 24-month-old rats for EM48 immunoreactive products (Fig. 3E–H). At the ages of 12 (Fig. 3G), 18 (Fig. 3A–D), and 24 months punctuate labeling was evident, which was most pronounced at the age of 24 months. No aggregates or inclusions were found in the brain of 1- and 6-month-old rats.

Postmortem concentrations of tryptophan and biogenic amines

Since altered tryptophan and dopamine metabolism is linked to HD, we examined neurochemical alterations in the transgenic HD rats using a highly sensitive HPLC method (23). Striatal dopamine levels were decreased only about 20% in heterozygotic rats whereas in homozygotic rats a reduction of nearly 80% was found (Fig. 4A). The levels of dopamine and DOPAC in the parietal cortex of homozygotic animals were not significantly changed (Fig. 4B, D and E). Tryptophan concentrations were decreased 2-fold in striatum (Fig. 4E), but not significantly different in parietal cortex (Fig. 4F). Interestingly, the levels of xanturenic acid were nearly depleted in the striatum of homozygotic transgenic rats (Fig. 4G) and undetectable in the parietal cortex (Fig. 4H). In contrast, in heterozygotes levels of xanturenic acid were elevated in the parietal cortex (Fig. 4H), but unchanged in the striatum (Fig. 4G). No significant changes in other neurotransmitter levels were found.

Focal lesions in the striatum, enlarged lateral ventricles, and reduced brain glucose metabolism

To examine whether transgenic animals display neuropathological signs detectable by magnetic resonance (MR) imaging, we performed MR investigations on 8-month-old homozygotic HD rats. MR scans revealed enlarged lateral ventricles (Fig. 5C and D) and focal lesions in the striatum (Fig. 5F).

Since clinical studies have consistently revealed reductions in striatal glucose metabolism, we studied the local cerebral metabolic rate of glucose (lCMRGlc) in transgenic rats using [18F]FDG (fluor-deoxy-glucose) and a high-resolution small-animal PET (positron emission tomography). PET studies were accompanied by ex vivo [18F]FDG measurements in order to test their reliability.

Harderian glands and different parts of the brain, such as olfactory bulb and caudato-putamen, were clearly distinguishable (Fig. 6). Individually co-registered MR images allowed a precise delineation of the whole brain as region of interest (ROI), as indicated by the red line (Fig. 6A and E). The defined ROI was measured in the co-registered PET image (Fig. 6B–D, F–H). Mean ICMRGlC values, as calculated from animal PET data of control animals, were 54.98±15.53 [μmol/ (100 g x min)] for the whole brain. Mean ICMRGlC values of hetero- and homozygotic animals were lower than control values (see legend of Fig. 6). Metabolic abnormalities of homozygotic animals were significantly different from controls (P < 0.05).

After completion of the PET scanning, we subsequently acquired ICMRGlC values ex vivo using [18F]FDG autoradiography (Fig. 6J and K). Similar to the in vivo situation determined by [18F]FDG-PET, mean ex vivo ICMRGlC values of homozygotic animals were significantly lower than control values (P < 0.05). A statistical comparison of autoradiographic
and animal PET data indicated that $\text{LCM}_\text{Glc}$ values were significantly similar ($P < 0.05$).

**DISCUSSION**

In this report we describe the first transgenic rat model for Huntington’s disease, which displays symptoms similar to the most frequent late-onset form of HD. It should be emphasized that these transgenic rats represent the first animal model of a human neurodegenerative disorder of the brain *per se* and that these animals express a high-end adult-onset HD allele, which is associated with a slow disease progression and pathology restricted to the striatum. Other symptomatic transgenic mice, however, express very large repeats that are only found in juvenile HD patients. Thus, these HDtg rats are especially useful for studying pathological changes that may be commonly present in the majority of adult HD patients, making this rat model more valuable than other mouse models in evaluating novel therapeutics on HD.

Transgenic rats develop slowly progressive phenotypes with emotional, cognitive, and motor deteriorations. The emotional disturbance is characterized by a reduction of anxiety, which resembles similar observations in R6/2 HD transgenic mice (16). Cognitive decline is also a feature of HD (24). Early in the course of HD, patients frequently show impairments of spatial working memory (25), and comparable deficits are also found in R6/2 mice (15,26) as well as in our HD transgenic rats. These data suggest a common underlying neuropathological mechanism in HD and corresponding animal models.

Neuropathological examination revealed nuclear inclusions and neuropil aggregates. EM48 labeled aggregates are mainly found in the striatum of transgenic rats at the age of one year and older. EM48 labeling shows a distribution pattern similar to that in the human condition (20). Similar results were previously reported in HD knock-in mice expressing full-length
mutant huntingtin under the endogenous mouse HD promoter (21,27). A remarkable observation in neurochemistry was that xanturenic acid was nearly completely depleted in the striatum and the parietal cortex. The levels of xanturenic acid were higher in the less afflicted heterozygotes, perhaps reflecting a neurochemical defense mechanism against the excitotoxicity of the overactive indoleamine (2,3)-dioxygenase pathway (28). Similar to HD patients, the levels of tryptophan were decreased in the striatum of homozygotes. Decreased DA and normal DOPAC levels are indicative of increased DA turnover. Decreased levels of tryptophan may be related to an increased formation of quinolinic acid, a neuroexcitatory molecule with neurotoxic properties (5). These findings support the hypothesis that both increased formation of quinolinic acid (28) and decreased production of neuroprotective metabolites from tryptophan (29) may be relevant to the pathogenesis of HD.

An important feature of the presented HD rat model is its suitability for in vivo metabolic and structural imaging, which cannot yet be achieved with transgenic mice. MR scanning demonstrated an enlargement of the lateral ventricles and focal signal abnormalities in the striatum of HD transgenic animals, although quantitative assessment of striatal neurons revealed no significant cell loss. This indicates that striatal atrophy depicted by MR imaging is rather a consequence of shrinkage than neuronal death. In high-resolution animal PET we found a significant reduction of brain glucose metabolism in 2-year-old homozygotic HD rats. In late stages of human HD, clinical PET studies consistently revealed reduced lCMR Glc in the striatum (30,31). Thus, this report provides evidence that the novel HD transgenic rat model does closely resemble the human pathological condition. It is suited for non-invasive in vivo investigations of brain metabolism and most probably of further in vivo parameters (e.g. receptor density, enzyme activity). Brain atrophy and extracranial tracer accumulation, however, necessitate the application of high-resolution tomographs and a careful evaluation of partial volume and spill over effects.

We report the successful development of a transgenic rat model of HD, which expresses a high-end adult onset HD allele with 51 CAG repeats and which exhibits a high degree of similarity to the most frequent adult type of the disease, thereby permitting in vivo monitoring of individual disease progression by high-resolution imaging (PET and MRI). For the first time it is now possible to follow up disease progression in longitudinal in vivo studies and to monitor the effects of long-term treatments, microsurgery, neuronal cell transplantation, or antisense approaches on discourse of experimental HD.

**MATERIALS AND METHODS**

**Generation of transgenic rats**

To generate the transgene construct, PCR was performed using DNA from a HD patient (19/51 CAGs) with Primer Hu 4.
(ATGGCGACCTGGAAAGCTGATGAA) and Hu3-510 (GGGCCCTGAGCTGAGCCAGC). This PCR product was subsequently digested with EcoRI. The first 154 nucleotides of the cDNA RHD10 containing nt 1–1962 of the rat HD gene (18) were removed by restriction of the clone with EcoRV and Eco81I. This fragment was replaced by the PCR product. Subsequently, a 885 bp rat HD promoter fragment from position −900 to −15 (19) was ligated upstream of the cDNA and a 200 bp fragment containing the SV40 polyadenylation signal was added downstream of the cDNA resulting in RHD/Prim51A. The insert was excised with XbaI and SspI out of the cloning vector and microinjected into oocyte donors of Sprague–Dawley (SD) rats (32,33). Tail DNA was extracted from each of the offspring and Southern blots of EcoRI digested DNA were performed to screen for founders.

For western blot analysis, frozen brain halves and dissected brain areas were homogenized and protein extracted. Protein extracts were subjected to SDS–PAGE and blotted electrophoretically onto Immobilon-P membranes. Detection of huntingtin protein was performed basically as described (34) using the polyclonal anti-huntingtin antibody 675.

Behavioral phenotyping of the HD transgenic rat line

The considerations for behavioral phenotyping of transgenic and knockout mice (35) were adapted with specific modifications for testing rats. All procedures were approved by the Government of Lower Saxony in Hannover, Germany, and performed in compliance with international animal welfare standards. The elevated plus maze (TSE-Systems, Bad Homburg, Germany) was equipped with light beam sensors and had two open arms (50 × 10 cm) and two enclosed arms of the same size. The experiment was conducted with 2-month-old rats as previously described (36). An increase of the time spent in the open arms is interpreted as an anxiolytic-like response. An automated sensor-equipped eight-arm radial maze (TSE) was used to measure learning and memory in an experimental design testing exploring behavior and working memory (WM) errors in allocentric orientation (37). An accelerating rotorod for rats (TSE) was used to measure fore- and hind-limb motor coordination and balance. Training consisted of three trials per day on four consecutive days. The duration of each trial was 5 min on accelerating mode of the apparatus. The maximal speed level and the mean latency to fall off the rotarod were recorded on three consecutive tests. The duration of each trial was 5 min on accelerating mode of the apparatus. The maximal speed level and the mean latency to fall off the rotarod were recorded on three consecutive tests.

Immunohistology and light microscopic examination

Brains of HD transgenic rats and controls at the age of 1, 6, 12, 18 and 24 months were perfused intracardially with PBS followed by paraformaldehyde and postfixed. Free-floating sections were pre-blocked in normal goat serum, Triton-X and...
avidin, and incubated with EM48 antibody (1:400 dilution) at 4°C for 24 h (20,21). The EM48 immunoreactive product was visualized with the avidin–biotin complex kit (Vector ABC Elite, Burlingame, CA, USA).

**Analysis of neurotransmitters from post-mortem tissue samples**

Tryptophan and its kynurenine, catechol- and indoleamine metabolites were measured by electrochemical HPLC, as described previously (23). Briefly, striatum and parietal cortex of 18-month-old transgenic HD rats were dissected, weighed and sonicated in perchloric acid. The homogenate was centrifuged and 20 μl of supernatant was injected into a HPLC system (ESA model 5600 CoulArray module, Chelmsford, MA, USA) with two coulometric array modules, each with four working electrodes. The chromatographic separation was achieved on an ESA MD-150 reversed-phase C18 analytical column with a Hypersil pre-column.

**MR scanning**

Rats were anesthetized with 2% isoflurane and fixed in a stereotaxic frame. MRI was performed on a 4.7 T Bruker Biospec scanner with a free-bore of 20 cm equipped with an actively RF-decoupled coil system. A whole-body birdcage resonator enabled homogeneous excitation, and a 3 cm surface coil was used as receiver. $T_2$-weighted spin echo images were acquired using a rapid acquisition relaxation enhanced (RARE) sequence (38). Eleven axial and seven coronal slices were measured (slice thickness: 1.5 mm axial; 1.3 mm coronal; field of view, 3.2 × 3.2 cm; matrix, 256 × 256; TR/TE 3000/19 ms; six averages).

**PET studies**

PET imaging was performed on a dedicated high-resolution small-animal PET scanner (`TierPET') as previously described (39) on 24-month-old homozygotic (+/+; $n = 6$) and heterozygotic animals (+/−; $n = 7$), as well as age-matched controls (−/−; $n = 6$). Reconstructed image resolution was 2.1 mm, which is homogeneously maintained throughout the entire field of view. A precise anatomical identification of rat brain regions was achieved by co-registration of magnetic resonance (MRI; Siemens Magnetom, 1.5T, equipped with a dedicated small limb coil) and PET images. Animals received an injection of 0.3 ml $[^{18}F]$FDG (1 mCi/ml, solved in NaCl 0.9%) under isoflurane sedation. After 30 min animals were anesthetized with ketamine/xylazine and glucose concentrations and input function were detected by serial blood samples. After a 60 min PET scan brains were removed and immediately frozen. Cryostat sections (20 μm) were exposed to a phosphor imaging plate (BAS-SR 2025, Fuji, Germany) together with calibrated fluorescent-18 brain paste standards. Imaging plates were scanned with a high-performance imaging plate reader (BAS5000 BioImageAnalyzer, Fuji, Germany; spatial resolution, 50 μm). Local cerebral metabolic rate of glucose (ICMR$_{\text{Glc}}$) was calculated on the basis of the operational equation used in 2DG autoradiography studies (40) with modified rate and lumped constants to account for the difference in kinetic characteristics between FDG and 2DG. The following constants (41) were used: $k_2 = 0.30; k_3 = 0.40; k_4 = 0.068$; lumped constant, LC = 0.60. Similarity of ICMR$_{\text{Glc}}$ as determined by FDG-PET and ex vivo autoradiography was analyzed by linear regression analysis.

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