Conference Program

Sunday, November 8

Arrival and check-in

16:00–18:00
Czech-Norwegian research program meeting

18:00
Registration, welcome drink and dinner

Monday, November 9

7:00–9:00
Breakfast, registration

Morning
Welcome/Introductory comments by organizers

9:00–9:10
Jan Motlik, Libechov, Czech Republic

Opening lectures

9:10–9:30
Monika Baxa, Libechov, Czech Republic
Living with Huntington's disease in the Czech Republic
The Czech Huntington's Disease Association (CHDA), its activity and cooperation with the Research Center PIGMOD

9:30–10:30
Douglas Macdonald, CHDI Foundation, USA
A strategy for the identification of translatable HTT lowering biomarkers

Scientific program of the Conference will be realized in the following sessions:

Session I
Neurodegenerative diseases, perspectives from rodent models to clinical
Chair: Zdenka Ellederova

10:30–11:00
Libo Yu-Taeger, Tubingen, Germany
Transgenic rat models for polyglutamine diseases

11:00–11:30
Huu Phuc Nguyen, Tubingen, Germany
Olesoxime suppresses calpain activation and mutant huntingtin fragmentation and ameliorates disease phenotypes in the BACHD rat

11:30–12:00
Coffee break

Session II
Emerging technologies and development of large animal models of disease
Chair: Martin Marsala

12:00–12:30
Nik Klymiuk, Munich, Germany
Pig models for monogenic diseases – how to make use of diverse gene targeting approaches for modifying the porcine genome

12:30–13:00
Pavlina Konstantinova, Amsterdam, Netherlands
Pre-clinical evaluation of AAV5-miHTT gene therapy of Huntington's disease

13:00–14:00
Lunch

Afternoon

Session III
Translatable measures: patients to large animal models and back
Chair: Jan Motlik

14:00–14:30
Martin Marsala, San Diego, USA
Experimental modeling and clinical treatment of amyotrophic lateral sclerosis by spinal grafting of human spinal stem cells

14:30–15:00
Stefan Juhas, Libechov, Czech Republic
Development and validation of brain and spinal cord vector and cell-delivery techniques in pre-clinical minipig models of neurodegenerative disorders

15:00–16:00
Marian Hruska-Plochan, Zurich, Switzerland
Modeling ALS on human neurons *in vitro*

16:00–16:20
S. J. Gadher, Life Technologies
Antibody-based investigational approaches in neuro-proteomics and neurodegenerative diseases

16:20–16:40
Hana Kovarova, Libechov, Czech republic
Reduction of IFNα and IL-10 in central nervous system and increase in peripheral Il-8 in transgenic porcine Huntington's disease model

16:40–17:00
Technical News

Jana Fukalova, BioTech
Think possible with Cytation

Milan Kopecek, Accela s.r.o
In vivo instruments for translational neuro-research

Session IV
17:00–19:00
Poster viewing with coffee

19:00–22:00
Dinner and wine tasting

Tuesday, November 10

7:00–9:00
Breakfast

Morning

Session V
Large animal model of HD
Chair: Douglas Macdonald

9:00–9:40
Emøke Bendixen
Pig Peptide Atlas: a tool for improved farm animal and model organism research

9:40–10:20
Zdenka Ellederova
Phenotype development of TgHD minipigs

10:20–10:50
Ralf Reilmann, Muenster, Germany
The TgHD minipig on the road to preclinical studies – current state of the art in behavioral and MR imaging assessments

10:50–11:20
Coffee break

11:20–11:50
Taras Ardan, Libechov, Czech Republic
Investigation of proteolytic enzymes expression in brain tissue and cultivated retinal pigment epithelial cells at transgenic animal model of Huntington´s disease

11:50–12:20
Tereza Tykalova, Prague, Czech Republic
Grunting in genetically modified minipig animal model for Huntington's disease – pilot experiments

12:20–12:50
V. Kruft, SCIEX, Germany
Digital quantitative image of any proteomic sample in the MS2 space: advances in data-independent LC/MS/MS acquisition strategies

12:50–14:00
Lunch

Session VI
Comparative studies of Huntington's disease
Chair: Lars Eide

14:00–14:30
Georgina Askeland, Oslo, Norway
Assessment of mitochondrial DNA damage in affected and peripheral tissue in Huntington´s disease and exploring the role of stem cell origin

14:30–15:00
Hana Hansikova, Prague, Czech Republic
Mitochondrial alterations in tissues with high energetic demand in minipig model transgenic for N- terminal part of human mutated huntingtin

Session VII
DNA repair in Huntington's disease models
Chair: Marian Hruska-Plochan

15:00–15:30
Arne Klungland, Oslo, Norway
DNA stability and Huntington's disease

15:30–16:00
Petr Solc, Libechov, Czech Republic
Double strand DNA breaks response in Huntington´s disease

16:00–17:00
Discussion Forum
Discussion leaders: Douglas Macdonald and Ralf Reilmann

Closing remarks: Jan Motlik
Coffee break

ABSTRACTS

A01 Living with Huntington's disease in the Czech Republic

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Key words: Czech Huntington Association – life with Huntington's disease

It is estimated that 700–1,000 patients with Huntington's disease (HD) and 4–5 times more people at risk live in the Czech Republic. As a rare disease in our country, HD is "unknown" for general public. Unfamiliarity and lack of understanding of HD, together with the hunger for help to HD families, lead to the foundation of the Czech Huntington Association (CzHA). CzHA provides support for patients with HD, people at risk and also for caregivers. Patients, their family relatives and friends welcome the recondition-educational weekend stays. These stays offer the possibility of tutorial discussions with neurologists, psychologists, psychiatrists, physiotherapists, ergotherapists and genetics specialized for HD. Persons at risk solve the question of genetic testing and preimplantation genetic diagnosis. Caregivers, who mainly are the family members of HD patient/s and often bear burdensomeness of HD aspects for several years, need not only educational lectures about the care for HD patients, but also welcome the psychological support and practicing of relaxation techniques. Periodical bulletin Archa brings the information about social and health care, and also about news in HD research field. Moreover, CzHA have published several brochures about living with HD. CzHA in collaboration with HD medical specialists train the personnel of residential facilities in specific aspects of caring for HD patients. CzHA presents a case of HD patients on meetings with health insurance companies and Ministry of Health of the Czech Republic. CzHA solves also problems of individuals eg. possibilities to obtain financial support for providing of health facilities and rehabilitations or problems with homelessness of HD patients. CzHA is member of international HD associations (International Huntington Association, European Huntington Association, European Huntington's Disease Network), EURODIS – Rare Diseases Europe and also Czech associations focused on supporting of disabled people. Since 2008, CzHA collaborates with Institute of Animal Physiology and Genetics (IAPG) in Libechov, Czech Republic. IAPG informs patients and their families about news in HD research field in plain language either on meetings or in bulletin Archa. CzHA aims to participate on generation of residential facility specialized for HD patients, fights against discrimination of the people at risk and aims to participate on HD research in collaboration with European Huntington's Disease Network. Despite of a huge CzHA's support, situation of HD community in Czech Republic – would still need a change. Excluding the efforts to improve the quality of life of HD patients and their families, CzHA would like to improve also awareness and knowledge about HD in Czech society.

A02 A strategy for the identification of translatable HTT lowering biomarkers

Macdonald D

CHDI Management/CHDI Foundation, Los Angeles, USA

Key words: Huntington's disease – biomarker – cerebrospinal fluid (CSF) – HTT lowering – HTT quantification – PET imaging

Huntington protein (HTT) lowering is a key therapeutic strategy for Huntington's disease (HD). Reducing the amount of the disease-causing expanded HTT protein in the brain of patients is predicted to slow the progression of the disease. Several approaches are being employed to lower HTT including antisense oligonucleotides and siRNAs, as well as gene therapy approaches using viral delivery of miRNAs, shRNAs, and zinc-finger repressor proteins. To enable the advancement of such therapeutics to the clinic, translatable proteomic, imaging, and physiological HTT lowering pharmacodynamic biomarkers are being explored using preclinical models of HD. We seek to identify and validate outcome measures that indicate that the delivery of a HTT lowering therapy does, in fact, lower the amount of HTT protein in the brains of HD patients.

A03 Olesoxime inhibits the formation of mutant huntingtin fragments through suppression of calpain activation and improves behavior and neuropathology in the BACHD rat

Nguyen HP[1], Clemens LE[1–3], Weber JJ[1,2], Wlodkowski TT[1,2,4], Yu-Taeger L[1,2], Michaud M[5], Calaminus C[6], Eckert SH[7] , Gaca J[7,8], Weiss A[9,10], Magg JC[1,2], Jansson EK[1,2], Eckert GP[7], Pichler BJ[6], Bordet T[5,11], Pruss RM[5], Riess O[1,2]

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Huntington's disease is a fatal human neurodegenerative disorder caused by a CAG repeat expansion in the *HTT* gene, which translates into a mutant huntingtin protein. A key event in the molecular pathogenesis of Huntington's disease is the proteolytic cleavage

of mutant huntingtin, leading to the accumulation of toxic protein fragments. Mutant huntingtin cleavage has been linked to the overactivation of proteases due to mitochondrial dysfunction and calcium derangements. Here, we investigated the therapeutic potential of olesoxime, a mitochondria-targeting, neuroprotective compound, in the BACHD rat model of Huntington's disease. BACHD rats were treated with olesoxime via the food for 12 months. *In vivo* analysis covered motor impairments, cognitive deficits, mood disturbances and brain atrophy. *Ex vivo* analyses addressed olesoxime's effect on mutant huntingtin aggregation and cleavage, as well as brain mitochondria function. Olesoxime improved cognitive and psychiatric phenotypes, and ameliorated cortical thinning in the BACHD rat. The treatment reduced cerebral mutant huntingtin aggregates and nuclear accumulation. Further analysis revealed a cortex-specific overactivation of calpain in untreated BACHD rats. Treated BACHD rats instead showed significantly reduced levels of mutant huntingtin fragments due to the suppression of calpain-mediated cleavage. In addition, olesoxime reduced the amount of mutant huntingtin fragments associated with mitochondria, restored a respiration deficit, and enhanced the expression of fusion and outer-membrane transport proteins. In conclusion, we discovered the calpain proteolytic system, a key player in Huntington's disease and other neurodegenerative disorders, as a target of olesoxime. Our findings suggest that olesoxime exerts its beneficial effects by improving mitochondrial function, which results in reduced calpain activation. The observed alleviation of behavioral and neuropathological phenotypes encourages further investigations on the use of olesoxime as a therapeutic for Huntington's disease.

A04 Transgenic rat models for polyglutamine diseases

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Key words: polyglutamine diseases – huntingtin – CAG repeats – transgenic rat model

Polyglutamine (polyQ) diseases are a group of genetic neurodegenerative disorders caused by a trinucleotide CAG repeat expansion in protein-coding regions of specific genes. To date, nine polyQ disorders have been described: Huntington's disease (HD), spinocerebellar ataxia (SCA) type 1, 2, 3, 6, 7 and 17, dentatorubropallidoluysian atrophy, and spinobulbar muscular atrophy. Knowing the single genetic cause of each disorder allows us to develop models that recapitulate many aspects of human disease. Rat models have made substantial contributions to our understanding of biological function and behavior, due to excellent learning abilities and relatively larger brain size compared to other small animal models. In our laboratory, we generated transgenic rats for HD (BACHD rats) and SCA17 (TBPQ64 rats).

HD is caused by an expansion of CAG repeats in gene huntingtin (*HTT*), characterized by motor, cognitive, and psychiatric deficits as well as neurodegeneration and brain atrophy beginning in the striatum and the cortex and extending to other subcortical brain regions. BACHD transgenic rats were generated using a human bacterial artificial chromosome (BAC), which contains the full-length *HTT* genomic sequence with 97 CAG/CAA repeats and all regulatory elements. These rats display a robust, early onset and progressive HD-like phenotype including motor deficits and anxiety-related symptoms. Neuropathologically, the distribution of neuropil aggregates and nuclear accumulation of N-terminal mutant huntingtin in BACHD rats is similar to the observations in human HD brains. In addition, reduced dopamine receptor binding and fractional anisotropy (FA) were detectable by *in vivo* imaging.

SCA17 is caused by an expansion of CAG repeats in the gene coding for TATA-box-binding protein (TBP), characterized by ataxia, dystonia, and seizures, dementia, psychiatric and extrapyramidal features as well as mild sensorimotor axonal neuropathy and cerebral and cerebellar atrophy. TBPQ64 rats carrying a full human cDNA fragment of the TBP gene with 64 CAA/CAG repeats show a severe neurological phenotype including ataxia, impairment of postural reflexes, and hyperactivity in early stages followed by reduced activity, loss of body weight, and early death. The severe phenotype of SCA17 rats was associated with neuronal loss, particularly in the cerebellum. Degeneration of Purkinje, basket, and stellate cells, as well as changes in the morphology of the dendrites, nuclear TBP-positive immunoreactivity, and axonal torpedos were found by light and electron microscopy.

A05 Pig models for monogenic diseases – how to make use of diverse gene targeting approaches for modifying the porcine genome

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Key words: somatic cell nuclear transfer – BAC-vector – animal models

Mouse models are standard tools for evaluating genetically inherited diseases, but for many of those it appeared that mutating the orthologous gene in the mouse does often not result in a phenotype that is comparable to that of human patients, either in the severity of the disease or in the pathogenesis or both. Even if mutations affect amino acid positions that are conserved between human and mouse, the result is often discouraging. Thus, it has been clear for a long time that alternative animal models are strongly demanded, but until recently, such models were dependent on the presence of spontaneously occurring mutations. With the development of somatic cell nuclear transfer (SCNT), however, a powerful tool became available that allowed the usage of genetically modified primary cells for generating animal models in species where embryonic stem cells were not available. First such models have been established by using additive gene transfer, but optimal monogenic disease models would definitely require a site-directed mutagenesis in primary cells. This turned out to be much more difficult for pig primary cells than for embryonic stem cells that are available for mouse our human.

Because conventional DNA-based vectors containing a few kb of homology to the target region were only efficient in single experiments, different groups were aiming at improving the rate of homologous recombination. While some researchers made use of AAV-based vectors, we focused on the usage of BAC-vectors for achieving the desired modifications, following the simple idea that extending the regions of homology to 100–200 kb would also increase interaction of genomic DNA with the exogenous vector. This was first successfully demonstrated by modifying the porcine *CFTR* gene for producing a model for cystic fibrosis as well as for the porcine *DMD* gene for generating a pig with Duchenne Muscular Dystrophy. Both models readily revealed a phenotype that was much more similar to that seen in human patients than the numerous mouse models are. By using this BAC-based approach we were not only able to modify the *CFTR* and *DMD* loci in different primary cells lines of both sexes, but we modified also other genomic regions in the pig genome, such as *GGTA1* or *POU5F1*, albeit in a context that is not related to monogenic disease research.

BAC-based approaches were sufficient for introducing genetic modifications in porcine cell clones, but it became soon apparent that the efficacy was too low to yield cell clones with modifications on both alleles at a considerable rate. Regarding the relatively long generation time of pigs, this would have been desirable, so we combined BAC vectors as well as conventional vectors with innovative designer nucleases such as zinc-finger nucleases (ZFN) or CRISPR/Cas9 and experienced a dramatic increase of homologous recombination up to 95% of all examined cell clones. We also succeeded in modifying gene loci by the combined usage of nuclease & vector in the rare cases we experienced an unsuccessful attempt with the BAC vectors alone. In addition, we also showed that nucleases alone can be used for deleting the function of a gene by introducing frame-shift mutations on both alleles in a single attempt.

Thus, the presently available tools already allow the efficient generation of tailored large animal models and future improvements will reveal their potential to introduce even more complex genetic modifications, such as humanization of entire genes.

A06 Pre-clinical evaluation of AAV5-miHTT gene therapy of Huntington's disease

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Key words: Huntington's disease – gene therapy – AAV5-miHTT – minipigs

Gene therapy is one of the most advanced approaches investigated for the treatment of Huntington's disease (HD). The most upstream therapeutic target in HD is the mutated huntingtin (HTT) and the goal is gene silencing with therapeutic miRNAs (miHTT) delivered with adeno-associated viral vector (AAV). Two major approaches have been undertaken for the development of RNAi-based gene therapy of HD: total HTT silencing by targeting exon 1 and allele-specific inhibition by targeting heterozygous SNPs linked to the mutant HTT. SNP rs362331 in exon 50 and SNP rs362307 in exon 67 were selected as they have the highest prevalence of heterozygosity in HD. The most efficient miHTT candidates were incorporated in AAV5 vectors and produced using the established uniQure baculovirus-based manufacturing platform. Proof of concept studies have shown efficacy of AAV5-miHTT in the lentivirally-derived HD rat model and the humanized Hu97/18 mouse model. In both models AAV5-miHTT delivery resulted in a lower concentration of the disease-inducing HTT protein associated with a delay of neurodegeneration and in reduction of mutant HTT aggregates. Direct intrastriatal delivery of AAV5-GFP by convection-enhanced diffusion (CED) injection or cerebrospinal fluid (CSF) delivery were evaluated in non-human primates (NHP) and minipigs to identify the best biodistribution profile for HD therapy. CED injection resulted in almost complete transduction of the NHP striatum and different areas of the cortex. Similarly, intrastriatal transduction of neuronal and glial cells of AAV5-GFP was observed in minipig putamen and caudate nucleus. In the minipig cortical areas mainly glial cells were transduced. Further studies in HD minipig model will aim to determine the level of HTT silencing in a large brain animals, the safety of the AAV5-miHTT approach and the long-term viral persistence. The miHTT processing and off-target potential will be determined to support the clinical development of the therapeutic candidate. AAV5-miHTT provides a huge therapeutic benefit for the HD patients as it will allow for life-long HTT suppression upon single vector administration.

A07 Experimental modeling and clinical treatment of amyotrophic lateral sclerosis by spinal grafting of human spinal stem cells

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Key words: rodent model of ALS – spinal neural precursors grafting – spinal-segment restricted ALS disease modeling

Twenty percent of familial cases of amyotrophic lateral sclerosis (ALS) are caused by mutations in superoxide dismutase 1 (SOD1) [1,2]. In the 20 years since mutation in SOD1 has been known to be causative of a proportion of inherited ALS, a consensus view has emerged that the mutations cause age-dependent degeneration and death of upper and

lower motor neurons from acquisition by mutant SOD1 of one or more toxic properties, not loss of enzymatic activity [3,4]. Importantly, mutant SOD1 protein expression in microglia and astrocytes significantly drives rapid disease progression [5,6], findings which have led to the conclusion that ALS pathophysiology is non-cell autonomous [3]. There are no effective therapies, but for SOD1 mutant-mediated disease suppressing synthesis of the mutant product or by improving local neurotrophic and glutamate buffering capacity (as achieved by spinal grafting of wild type neural precursors) in the local spinal cord milieu has been extensively explored as a possible treatment strategy. Our group, as well as others, have demonstrated a positive treatment effect after spinal lumbar grafting of clinical grade human spinal stem cells in rat SOD1 model (G93A) of ALS [7]. The data from the rodent efficacy studies, as well as safety studies, which employed pig model [8] of spinal cell grafting led to a Phase I safety trial in human ALS patients. The preparation of Phase II/B is currently in progress. One of the limitations of the rodent SOD1^{G93A} model is its aggressive nature, which is characterized by a rapid progression from the disease onset to end-stage. These properties precludes an effective utilization of this model to demonstrate treatment efficacy particularly when a long-term survival is required to achieve a full therapeutical potential. More specifically, on average 4–6 months (or longer) is required for grafted human spinal stem cells to mature and to acquire the differentiation phenotype which is consistent with a fully mature and functional CNS tissue including expression of mature neuronal and glial markers such as NSE (neuron-specific enolase), synatophysin and GFAP (glial fibrillary acidic protein-astrocyte marker). To address this issue we have recently developed a spinal segment(s)-restricted ALS model by overexpression the human mutated *SOD1^{G93A}* gene in lumbar spinal cord of naïve Sprague-Dawley rat. To achieve that, we have developed a novel technique of subpial AAV9 delivery. This technique, in contrast to intrathecal delivery, leads to a deep parenchymal AAV9 penetration and resulting transgene expression in the majority of neurons in dorsal and ventral horn of AAV9-injected segments. Over-expression of the human mutated *SOD1^{G93A}* gene in lumbar spinal cord led to a progressive deterioration of lower extremity motor function (paraplegia) at periods between 6–12 months after the *SOD1^{G93A}* gene delivery. The loss of neurological function corresponded with over 80% loss of lumbar A-motoneurons and appearance of misfolded SOD1 protein aggregates throughout the AAV9-injected region. Except of lower extremity paralysis, animals display normal upper extremity function, breathing and feeding behavior. This data demonstrates that this spinal segment(s)-restricted ALS model may represent an alternative rodent ALS model to study the long-term engraftment of neural precursors or alternatively can also be used to test gene-silencing and/or gene editing-based treatment strategies. In addition to the rodent model, the development of spinal regional ALS model in adult pig is currently in progress.

References

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A08 Development and validation of brain and spinal cord vector and cell-delivery techniques in pre-clinical minipig models of neurodegenerative disorders

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Key words: minipig models of neurodegenerative disorders – brain and spinal cord cell delivery techniques – subpial and intraparenchymal brain and spinal cord AAV5 and AAV9 vector delivery

The use of large animal models represents an essential component in preclinical development of cell-replacement and gene-delivery-based therapies. Over the past decade using a well-established strain of miniature pigs, we have developed several models of neurodegenerative disorders including a transgenic model of Huntington's disease, a chronic spinal cord traumatic injury model, and a model of transient global cerebral ischemia. Using a chronic spinal trauma model and a model of transient global cerebral ischemia, we have extensively tested the FDA-approved spinal and brain cell-injection devices and have defined the safety and optimal cell dosing of human clinical-grade neural precursors to be used in human clinical trials for treatment of ALS and chronic spinal trauma patients. Data from these studies were used in the approved IND applications (Neuralstem, Inc.) with both human clinical trials currently in progress. More recently, we have developed a novel technique of subpial AAV9 delivery. This new technique was extensively validated in rats and minipigs and has proven to be superior over the currently employed AAV delivery techniques (intrathecal or intra-ventricular) in terms of more robust parenchymal transgene expression and retrograde infection of supraspinal brain motor and sensory centers. This technique is currently being tested for its efficacy in silencing of the mutated human *SOD1* gene in a rat G93A model of ALS.

If successful, our future studies will focus on establishing large animal (pigs and non-human primates) safety data to enable prospective use of this vector delivery technique in human ALS and spinal trauma patients.

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A09 Modeling ALS on human neurons *in vitro*

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Key words: amyotrophic lateral sclerosis – *C9ORF72* – dipeptide repeat proteins (DPRs) – TDP-43 – human iPSC-derived NSCs – human chemical-induced neuronal cells (hciNs)

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease, which affects motor neurons leading to progressive paralysis and death within a few years from onset. ALS is genetically, pathologically and clinically linked to another neurodegenerative disease called frontotemporal dementia (FTD), which is the most common type of dementia below 60 years of age and is characterized by language and behavioral dysfunction. Both disorders are devastating and cause death within a few years from diagnosis.

Recently, intronic GGGGCC hexanucleotide expansion in an uncharacterized gene called *C9ORF72* was found to be the most common genetic cause of both ALS and FTD confirming the hypothesis that ALS and FTD represent opposite ends of the same disease spectrum. In addition to the typical TDP-43-positive inclusions, *C9ORF72* patients also develop inclusions that lack TDP-43, but contain abnormal dipeptide proteins (DPRs) that are produced by the intronic repeat RNA through an unconventional type of translation, called repeat-associated non-ATG (RAN) translation. Moreover, the hexanucleotide repeat RNA forms nuclear foci within neurons and glia of *C9ORF72* ALS patients.

Nevertheless, vast majority of ALS and FTD cases are sporadic with unknown etiology and therefore difficult to model. Accordingly, until very recently, research has been limited to the use of post-mortem patient samples. However, the recent advent of reprogramming technologies allow us to use accessible cells, such as fibroblasts, to generate patient-specific neurons and thus study the mechanisms of sporadic ALS *in vitro*.

Here we employ iPSC and hciNs (human chemical-induced neuronal cells) technologies to study both sporadic and *C9ORF72*-induced ALS. In addition, human iPSC-derived NSCs stably expressing DPRs were developed to dissect the pathogenic mechanisms of *C9ORF72* mediated ALS/FTD.

A10 Antibody-based investigational approaches in neuro-proteomics and neurodegenerative diseases

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Key words: neurodegeneration – disease models – multiplexing – antibodies – biomarker(s) – tau protein

Neurodegenerative diseases are devastating and affect millions of individuals worldwide. Unfortunately, no drugs are currently available to halt their progression, except a few that are largely inadequate. Genetic as well as environmental factors have been shown to be associated with neurological disorders and trigger many molecular and cellular alterations leading to progressive loss of neural cells and neurodegeneration. The advances in developing effective disease-modifying drugs as well as diseases biomarker(s) identification critically depend on understanding of the mechanisms of disease pathogenesis at molecular level.

The recent advances in proteomics and proteomic related technologies could significantly contribute to revealing the underlying molecular mechanisms as well as in the identification of novel biomarker(s) suitable for use in (pre)clinical trials for such neurodegenerative diseases. An ideal biomarker(s) would allow the mapping of mechanisms of action and its quantification could reflect treatment intervention in disease models and particularly in patients. Such biomarker(s) could come from measurements of analytes in serum, plasma or cerebrospinal fluid and may fulfill the criteria for a high throughput, high sensitivity and yet a low cost assay for the disease.

A number of methodologies including antibody independent targeted mass spectrometry quantification, enzyme-linked immunosorbent assay (ELISA), as well as Luminex xMAP 'bead-based' multiplexing technology are being utilised in developing new assays for monitoring of disease progression and novel therapeutic interventions or strategies. Here, we give examples of: 1. a quest for candidate biomarkers for Huntington's disease, 2. neural stem cell research with a view to cell replacement therapies as a promising strategy for neurodegenerative diseases, and 3. recently launched neuro panel with a selection of capture antibodies to facilitate measurements of Aβ levels; including Aβ1-40 and Aβ1-42 and total tau protein in various biological fluids.

Antibody-based investigational approaches can be powerful in facilitating 'cutting-edge' research in specialist areas of neuro-proteomics, stem cell research and biomarker discovery for neurodegenerative diseases.

A11 Reduction of IFNα and IL-10 in central nervous system and increase in peripheral IL-8 in transgenic porcine Huntington's disease model

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Key words: porcine Huntington's disease model – IFNα – IL-10 – IL-8 – multiplexing – cerebrospinal fluid – microglial cells

Huntington's disease (HD) is an inherited neurodegenerative disorder which is progressive and fatal. Any preventive or disease-modifying therapies are not available as yet. Although the pivotal role of neuroinflammation and activation of immune response is proven in mutant huntingtin (mtHtt) carriers, further studies are needed on innate and adaptive immune responses to mtHtt in both central nervous system (CNS) and peripheral system and their interconnectivity Additionally, cerebrospinal fluid (CSF) and blood plasma/serum appear to be a promising source of potential biomarkers for monitoring HD progression and efficacy of novel therapeutic strategies.

In our study we utilised a novel transgenic TgHD porcine model to investigate inflammatory and immune responses using bead based multiplexing Luminex xMAP technology. Among the most pronounced changes was the decline of IFNα in CSF of TG animals and this was observed from very early time intervals of HD. IFNα was also decreased in secretome of microglial cells but not blood monocyte in TgHD animals. In addition, IL-10 was lower in CSF as well as microglia secretome. On the contrary, elevated levels of pro-inflammatory IL-1β and IL-8 were produced by microglial cells of TgHD animals. Compared to several cytokine alterations observed in representative parts of CNS, only IL-8 was elevated in peripheral system in serum of TgHD minipigs. We further demonstrated higher proportion of the mtHtt related to endogenous Htt in microglial cells compared to blood monocytes in transgenic minipigs which may have a causative impact on cytokine production.

Observed dysregulation of cytokine profiles indicated neuroinflammation and possible lack of adaptive immune response in CNS whilst sign of inflammation was detected in peripheral system in TgHD porcine model. We revealed emerging role of IFNα and IL-10 in central nervous system inflammation and immune response imbalance in HD progression.

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A12 Phenotype development in TgHD minipigs

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Key words: phenotype – minipig model of Huntington's disease – reproductive failure – aggregates – phosphorylation – N-terminal fragments – motoric and behavioural impairment

In July 2009, the first transgenic minipig expressing N terminal part of human huntingtin (TgHD) was born. The transgenic minipig model was generated using microinjection of a lentiviral vector.

Mutated human *HTT* gene with 124 CAG repeats was incorporated into chromosome 1 (1q24–q25). Further analysis showed that the insertion of the lentiviral construct did not interrupt any coding sequence. In this study, we compared TgHD and wild type (WT) siblings in order to describe the phenotype development of TgHD minipigs.

Even though the neurological phenotype of Huntington's disease (HD) patients is the most prominent, the first sign of phenotype development in TgHD boars of F1 generation was a reproductive failure, starting at the age of 13 months. In further studies, we showed that sperm and testicular degeneration of TgHD boars is caused by gain-of-function of the highly expressed mutated huntingtin (mtHtt) in sperms and testes. Nevertheless, the HD is associated with neuronal death and the formation of aggregates in basal ganglia and cerebral cortex. Therefore, we focused on aggregate formation in the brain. Unfortunately immunohistochemical analysis could detect just a few spots resembling to aggregates in coronal sections of 36 months old TgHD, and none in WT. However, the filter retardation assay showed retention of higher molecular weight Htt polymeric structures in TgHD brains. Moreover, velocity and equilibrium sedimentation method also revealed mutated huntingtin (mtHtt) in higher insoluble fractions. Interestingly, mtHtt in higher insoluble fractions was not phosphorylated on Ser13 and 16. This is in accordance with published data that unphosphorylated rather than phosphorylated huntingtin on Ser 13, 16 tents to form aggregates.

Since previous studies showed that even neurons without aggregates undergo degeneration and death, we focused on the presence of N-terminal fragments. We screened several tissues for fragmentation, and found the most in cortex, and brain of TgHD minipigs.

Moreover, we performed behavioral and motoric tests of F0, and F1 generation of TgHD. Mainly the five years old boars show wobbly movements of their back legs, giving us hints of HD manifestation in transgenic minipigs around five years of age.

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A13 The TgHD minipig on the road to preclinical studies – current state of the art in behavioral and MR imaging assessments

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Key words: animal models – minipig – Huntington's disease – phenotyping – magnetic resonance imaging – imaging – behavioral – preclinical research

The TgHD minipig may fill an important gap in translational preclinical research between rodents and humans. The model can already be applied for studies targeting delivery or distribution of therapeutics. In addition, studies targeting proof-of-concept of certain mechanisms-of-action are possible. Lowering of mHTT expression, for instance, could be investigated with repetitive *in vivo* access to biospecimen in CSF, blood and tissue samples. For further translational research the availability of sensitive and meaningful phenotypical assessments is warranted. The presentation will review the current status of development of a battery of assessments that have been developed in the setting of the "TRACK-TgHD-Minipig" study. The study follows a cohort of TgHD and wild type minipigs with repetitive assessments including behavioral, motor, cognitive, and a variety of magnetic resonance imaging (MRI) endpoints. Motor assessments include the GAITRite gait analysis, a hurdle test, and a tongue coordination test. A color discrimination test was established to test cognitive function and a dominance test is applied to assess social hierarchy. Imaging modalities established allow volumetric analyses, fiber tracking and *in vivo* measurement of metabolites using MRI spectroscopy. Tools needed to further improve reliable imaging analyses such as a brain atlas for the Libechov minipig are in development and closed to finalization. The current state of development allows us to conclude that behavioral and imaging assessments of TgHD minipigs are feasible and can reliably be conducted repetitively in the setting of a longitudinal follow-up study. As the "TRACK-TgHD-Minipig" study has a "dynamic" protocol, tests may be revised in future assessments to allow for further learning as the study progresses. The translation to preclinical studies using the model is in progress and examples for applications of the model currently considered will be discussed same as further perspectives for applications of the assessments, e. g., in HD knock-in minipigs.

Acknowledgement: The "TRACK-TgHD-Minipig" study is funded by the CHDI Foundation.

A14 Investigation of proteolytic enzymes expression in brain tissue and cultivated retinal pigment epithelial cells at transgenic animal model of Huntington's disease

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Key words: Huntington's disease – transgenic porcine model – proteolytic enzymes

One of the key mechanisms in the pathogenesis of Huntington's disease (HD) is a proteolysis of mutant huntingtin (mtHtt). Previous studies revealed that small proteolytic fragments derived from mtHtt have particular cytotoxic characteristics. These fragments are highly toxic to neurons, which are located in the striatum and cortex, and inhibition of proteolysis of mtHtt significantly reduces neurotoxicity. Therefore, proteolytic cleavage as a source of these breakdown products was considered as an early or initial step in HD pathogenesis. In this study, we investigated the expression of proteolytic enzymes from the families of caspases, matrix metalloproteinases (MmPs), kallikreins and calpains on the transgenic minipig model of HD. For all investigations, we used WT and TgHD minipigs for N-terminal part of the human mtHtt (548aaHTT-145Q, both F2 generation, age 36 months; F3 generation, age 48 months in additional experiment), in some cases R6/2 mice were used as positive controls. Htt and proteases were examined immunohistochemically (IHC) and by immunofluorescence (IF) on cryostat sections, or biochemically by Western blotting (WB) using the following primary antibodies: anti-caspase-3, anti-caspase-8, anti-MmP-9, anti-MmP-10, anti-kallikrein-10, anti-calpain-5. Using IHC and WB, we demonstrated significantly increased expression of caspase-3 in nucleus caudatus and cortex area of TgHD minipigs in comparison to WT animals. MmP-10 expression was detected immunohistochemically in all brain structures of both WT and TgHD pigs, when the mildly increased expression was seen in the caudate nucleus of TgHD minipig. Increased MmP-9 expression was detected immunohistochemicaly in the striatum and cortex of TgHD minipig, and also biochemically were showed increased levels of processed forms of MmP-9 in cortex and cerebellum of TgHD minipig. Likewise, elevated levels of MmP-9 were observed by IF in retinal pigment epithelial cells (RPE) of TgHD minipig (48m). Calpain-5 was highly expressed in the striatum and cortex of TgHD and WT animals without differences between them. Even if the most proteolytic enzymes revealed the same or increased expressions in TgHD brains, the decreased expression of kallikrein-10 was detected in these brains in comparison to WT brains. In conclusion, we suggest that high levels of proteolytic enzymes detected in TgHD minipig can increase production of mtHtt derived proteolytic fragments and thus contribute to the disease development.

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A15 Grunting in genetically modified minipig animal model for Huntington's disease – a pilot experiments

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Key words: Huntington's disease – grunting – transgenic pigs – animal models – voice and speech disorders

Huntington's disease (HD) is an autosomal-dominant neurodegenerative disorder characterized by impairment of voluntary and involuntary movements, behavioral disorders and cognitive decline. Besides the main motor symptoms, voice and speech disorders have been documented in large majority of patients with HD. Slight changes in voice and speech production have also been observed in persons with preclinical stages of HD. The animal model of pigs is often used in preclinical studies. Although there are obvious differences in anatomy of articulation organs between pigs and humans, the same trends in pathophysiology mechanism can be expected in both grunting and human phonation. The main aim of the study was therefore to design a suitable experiment that would allow acquisition of a sufficiently long recording of grunting from as many pigs as possible. The second goal was to perform the final version of experiment in all available pigs and to evaluate the amount and quality of gained recordings. The database consists of 17 HD transgenic minipigs and 16 healthy siblings. Tested variants of the experiment, performed on subgroup of 4 sows, were divided into four subgroups: (a) positive – feeding, (b) positive – sound stimulation, (c) negative – hindering in movement, (d) negative – unpleasant touch. The evaluation of quality of elicited recording was performed using audio software where pure pig's grunting was selected and all acoustic artefacts deleted. The best results were reached using experiment where: 1. a recording device is put on pig's body, 2. pig is left alone for few minutes in the pen in order to calm down, and 3. person enters the room and tries to offer the pig feed while reversing. As a result, pig follows the person and grunts. Notably, it is useful to perform experiment with hungry pigs (omitting two feeding doses). Sufficiently long (20 single grunts or more) and clear recordings were received from 24 out of 33 pigs (73%). Our experiment is designed to be applicable to both genders and various ages and thus might be successfully used for gaining of pig grunting in future research focused on longitudinal investigation of possible disturbances in pigs' vocalization due to the HD.

Acknowledgement: The study was supported by the Czech Science Foundation (GACR 102/12/2230), Czech Technical University in Prague (SGS 15/199/OHK3/3T/13) and Charles University in Prague (PRVOUK-P26/LF1/4). This work was also supported by Program Research and Development for Innovation Ministry of Education, Youth and Sports ExAM CZ.1.05/2.1.00/03.0124.

A16 Digital quantitative image of any proteomic sample in the MS2 space: advances in data-independent LC/MS/MS acquisition strategies

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Recent advances in high resolution mass spectrometry, specifically QqTOF technology, have made it possible to acquire qualitative and quantitative information simultaneously from highly complex samples. The extreme speed and sensitivity of current instrumentation allows near complete analysis in information dependent (IDA) experiments. However, the concept of data independent acquisition (DIA) can now also be realistically applied for the first time. This will avoid the bias introduced by precursor selection and thus increase the reproducibility and comprehensiveness of data collection. In this data independent workflow – called MS/MS^{ALL} with SWATH™ acquisition – the Q1 quadrupole is stepped at defined mass increments across the mass range of interest, for example passing a 25 amu window into the collision cell independent of the number of precursors. Fragments – so called product ions – are analyzed in the TOF MS analyzer at high resolution. Due to the high speed of the QqTOF, these experiments can be done in a looped fashion at a cycle time compatible with LC separations. Post-acquisition MRM-like analysis can be performed on such datasets by the generation of large numbers of high resolution XIC's. These are identified and quantified by comparison to the available proteomic or MRM spectral databases.

Recent developments of the data-independent SWATH™ workflow will be explained and compared to other quantitative techniques in systems biology like quantification via SRM/MRM. These include the introduction of variable window sizes and increasing the number of programmable windows to add even more selectivity to the workflow – as well as the automatic removal of interferences, a SWATH™ specific feature that further increases the specificity and accuracy of the quantitative results. Finally, the OneOMICS™ cloud environment, that allows fast data processing and comparison of proteomics data with genomic and metabolomic datasets in the public domain, will be introduced.

Recently published investigations will be highlighted, including studies of kidney disease, histone modifications and the proteome of *M. tuberculosis*.

A17 Assessment of mitochondrial DNA damage in affected and peripheral tissue in Huntington's disease – possible role of stem cell origin

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Key words: Huntington's disease – mitochondria – DNA damage – minipigs

Huntington's disease (HD) is invariably coupled to DNA stability. Repair of nuclear DNA (nDNA) damage as well as prevention of mitochondrial DNA (mtDNA) damage determines disease progression in HD mouse models. Thus, DNA damage represents a potential diagnostic marker for monitoring disease and assessing therapy. However, affected tissue is not easily accessible and it is enigmatic how DNA integrity in the periphery correlates with that in affected tissue.

The aim of the current project is to identify biomarkers in easily accessible tissue that can be used to monitor disease. Using in-house established and published methods, we compared the integrity of nDNA and mtDNA in various tissues from the TgHD pig model with those obtained from HD patients and matching controls. The results demonstrate strong tissue-specific differences in mtDNA and nDNA integrity in the TgHD pig model, while no effects were identified in pig leukocytes. In contrast, samples from human patients demonstrate significant differences in mtDNA as well as nDNA integrity in leukocytes. A strong correlation was also seen between nDNA damage and copy number in pig leukocytes and spleen.

We are currently using the same material to evaluate how DNA integrity correlates with nuclear CAG somatic expansions and mitochondrial function. The impact of HD on peripheral tissue suggests that genotoxicity is not limited to affected areas, but may as well be influencing neurogenesis important for repopulation in the striatal region. We will test the idea that systemic (mt)DNA damage and the subsequent repair impairs CAG triplet stability in neural stem cells.

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A18 Mitochondrial alterations in tissues with high energetic demand in minipig model transgenic for N-terminal part of human mutated huntingtin

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Key words: mitochondria – respiratory chain – transgenic minipig model

Huntington's disease (HD) is neurodegenerative disorder caused by an abnormal expansion of CAG repeat encoding a polyglutamine tract of huntingtin (Htt). It has been postulated that mitochondria dysfunction and oxidative stress may play significant roles in the aetiology of the HD. Given the ubiquitous expression of Htt, all cell type with high energetic demand may be at risk for HD related dysfunction.

The aim of the present work was phenotypic monitoring of the mitochondrial functions and the detection of mitochondrial dysfunctions in tissues with high energetic demand in transgenic minipigs (TgHD) of F2 generation during 12–48 months of life.

Respiratory chain complexes (RCC), Krebs cycle enzyme, pyruvate dehydrogenase (PDH) activity and amount were analyzed by spectrophotometric, radioisotope and imunoelectrophoretic methods in TgHD minipig brain, heart and muscle homogenate and isolated mitochondria. Respiration was measured by polarography. Mitochondrial energy generating system capacity was characterized by oxidation rate of labelled substrates.

Decreased activity of complex I of RCC and PDH in TgHD frontal cortex was found in the age of 36-month-old contrary to 24-month-old animals. Amount of nutrient and stress sensor O-GlcNAcase was decreased in heart homogenates of in the age of 36-month-old TgHD minipigs. Although respiration of skeletal muscle mitochondria from 36-month-old HD minipigs was equal to control values, one year later the respiration after succinate and ascorbate + TMPD addition in 48-month-old HD pig's mitochondria was decreased to about 60% of control values. Furthermore, decreased oxidation of succinate to 60% and lower amount of RCC in 48-month-old HD was detected in comparison with WT. Our results indicate for the preclinical signs of HD in the studied tissues of TgHD minipigs.

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A19 DNA stability and Huntington's disease

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Key words: Huntington's disease – CAG repeats – DNA repair

Huntington's disease (HD) was first described by Johan C. Lund and Georg Huntington during the late 19th century. One of the major breakthroughs in the understanding of HD came with the identification of a gene containing a trinucleotide repeat that is expanded and unstable in the patients (The Huntington's disease collaborative research group, Cell 1993; 72: 971–983). HD is a genetically determined neurodegenerative disorder, for which onset is known to depend upon the length of glutamine-encoding CAG-repeat sequences lying within the Huntingtin gene. Recently, dramatic somatic CAG expansions have been detected in diseased human brains and in transgenic HD mice (Kennedy et al., Hum Mol Genet 2003; 12: 3359–3367). Yet, the mechanism of expansion remains poorly understood. We, and others, have identified a surprising role for DNA repair in triplet stability. Oxidative damage has long been associated with ageing and neurological disease; however, mechanistic connections of oxidation to these phenotypes have remained elusive. We have demonstrated that the age-dependent somatic mutation as-

sociated with HD in mice occurs in the process of removing oxidized base lesions, and is modulated by the base excision repair enzyme, 7,8-dihydro-8-oxoguanine-DNA glycosylase. The presentation will present current models for somatic CAG instability as analyzed on Huntingtons transgenic mice.

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A20 Double strand DNA breaks response in Huntington's disease

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Key words: Huntington's disease – DNA damage – double strand DNA breaks

There are strong evidences that DNA damage response (DDR) signaling significantly underline the molecular pathology of polyglutamine (polyQ) diseases, including Huntington's disease (HD). Double strand DNA breaks (dsDNA breaks) are the most deleterious DNA lesions. The can arise from own cell metabolism producing oxygen free radicals, replicative stress or also through transcription. After dsDNA breaks arise in cell, histone H2AX is rapidly phosphorylated on Ser-139 forming γ-H2AX. This crucial histone modification is mediated by ATM action, although other members of PI3/PI4-kinase family ATR or DNA-PK can do it as well. It was shown that dsDNA breaks are an early event in HD pathology. In mouse R6/2 HD model γ-H2AX is elevated in striatal neurons from 4-weeks-old mice. Interestingly, γ-H2AX increases both in wild type and R6/2 mice during age, however γ-H2AX is significantly higher in R6/2 than in wild type animals. Furthermore, striatal neurons and fibroblast of HD human patients exhibit higher γ-H2AX level. In our work we are analyzing primary fibroblasts from TgHD minipigs to answer the question whether mtHtt does increase number of dsDNA breaks in TgHD minipig model. This knowledge will be useful for using DNA damage markers as a way how to monitor effectivity of mtHtt lowering based therapy. Moreover, experimental targeting of DDR signaling proves therapeutic potential for HD at least in cell based and small animal models. Our preliminary data from minipigs shown significant decrease in activatory phosphorylation of ATM and ATM-dependent p53 phosphorylation after exogenous induction of dsDNA breaks. It suggests that ATM-p53 pathway is somehow compromised. Additionally we detected fewer DNA damage foci using 53BP1, γ-H2AX and MDC1 in mHTT cells after exogenous dsDNA breaks induction.

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POSTERS

P01 Behavioral and motoric testing of transgenic minipigs – focus on F0, F1, and F2 generations

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Key words: involuntary movements – behavioral tests – minipig model of Huntington's disease

Huntington's disease (HD) is an autosomal dominant monogenetic neurodegenerative disease caused by a pathological CAG expansion in exon 1 of the huntingtin gene leading to the production of the mutant huntingtin protein. The onset of the disease in patients is usually in mid-thirties. It is characterized by involuntary chorea like movements, poor balance, slurred speech, difficulty swallowing, thinking (cognitive) difficulty, and personality change. Interestingly, not all symptoms are experienced by all patients. One of the most used HD animal models has been R6/2 mouse. R6/2 also exhibit difficulties in a number of tasks namely swimming, beam traversing, and maintaining balance on the Rota-rod at the fastest rotating speeds. They have characteristic clasping when taken by tail.

In this project, we performed behavioral and motoric tests of our oldest minipigs expressing N-terminal part of human mutated huntingtin (TgHD), and their wild type (WT) siblings.

We implemented a variety of behavioral tests, including tunnel test, hurdle, seesaw, skittles, cover pan, and crossing a longitudinal and cross stepper. We observed higher fear or inability to perform certain tests, and wobbly movements of back legs of some mainly F0 and F1 TgHD animals. Scoring table of performed repeated tests was done. The obtained data were processed statistically.

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P02 Immunoelectrophoretic analysis of mitochondrial protein status in skeletal muscle of minipigs transgenic for the N-terminal part of human mutated huntingtin

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Key words: Huntington's disease – large animal model – skeletal muscle – mitochondria

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by the expansion of CAG repeats on chromosome 4p16.3, which results in elevated polyglutamine tract in huntingtin (Htt). Numerous studies demonstrated that CNS and peripheral pathogenic processes in HD share important features. Myocytes, similar to neurons, are post mitotic, long-lived cells, and muscle tissue is a tissue that is affected in HD patients by progressive wasting. Mutant Htt has been implicated in disruptions of mitochondrial functions whose impairment may contribute to the pathogenesis of HD.

The aim of our study was to monitor the level of mitochondrial proteins and their age-related changes in skeletal muscle of transgenic minipigs (TgHD) in F2 generation in age of 24, 36 and 48 months.

To investigate the native state of oxidative phosphorylation (OXPHOS) complexes, isolated mitochondria were separated by 6–15% BN-PAGE followed by Coomassie Brilliant Blue staining. Decrease native levels of complex I, III, IV and complex V were detected in dependence on age of minipig models. Pronounced reduction of complex III and complex V were revealed especially in 48m TgHD skeletal muscle samples. Isolated mitochondria were also separated by 12% SDS-PAGE followed by western blot. The resulting immunoblots were incubated with specific antibodies against selected mitochondrial proteins (Abcam) include OXPHOS complex subunits, pyruvate dehydrogenase (PDH) subunits, Krebs cycle enzymes and other mitochondrial proteins. Mild decrease in level of selected proteins were observed (Aconitase, PDH E2/E3 bp, COX1, COX5a), however intensity of changes were not the same for all individuals of the same age TgHD.

Studies of potential deregulation of the target proteins in peripheral tissues such as skeletal muscle could provide valuable information about biological changes that track disease progression.

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P03 Striatal magnetic resonance spectroscopy of transgenic HD minipigs

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Key words: striatum – magnetic resonance spectroscopy – transgenic – Huntington's disease – animal model – minipig

Our study is the first phenotyping study on TgHD minipigs from the Institute of Animal Physiology and Genetics in Libechov (Czech Republic). MRI scans including anatomical, diffusion-weighted and spectroscopic sequences were performed to assess anatomical and metabolic changes in the brain. Proton magnetic resonance spectroscopy (MRS) is a validated method for determining changes in the relative molecule concentration in Huntington's disease (HD) patients and in TgHD mice.

For determining relative molecule concentrations in the striatum of the TgHD and wild type minipigs we performed a baseline and two follow-up assessments. Feasibility and tolerability same as between group differences and longitudinal changes will be assessed.

Methods and techniques: The minipigs were scanned on a 3T Philips Achieva system at baseline, at one year and two year follow-up. Single-voxel based spectroscopy (PRESs) of the striatum on the right and left hemisphere was acquired using the following parameters: TR: 2 000 ms, TE: 32 ms, measured voxel size: 8 × 22 × 8 mm, averages: 176, scan duration: 6 min. The concentration of N-acetylasparate, myo-inositol, glutamate and glutamine relative to creatine was calculated by LCModel.

In the data collected no significant differences were seen between the transgenic and the wild type group at MRI 1 (baseline – 6 month of age) and 2 (18 months of age). The spectra showed well-resolved lines of the major metabolites, which were observed at similar relative concentrations as expected for humans. The data analysis for MRI 3 (30 month of age) is pending and the statistical analysis will be presented on the Conference on Animal Models for Neurodegenerative Diseases 2015.

In this pilot study cross-sectional baseline and longitudinal data from TgHD minipigs and controls was successfully acquired. Thus longitudinal changes between transgenic and wild type may be expected later on due to the slowly progressive nature of HD.

Acknowledgement: Founded by the CHDI Foundation.

P04 Spermatozoa immunophenotype markers associated with porcine HD model

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Key words: Huntington's disease – transgenic porcine model – spermatozoa

Huntington's disease (HD) is inherited and incurable progressive neurodegenerative disease. Predominantly rodent animal models are utilized to explore the mechanism and the potential treatment of HD. Large animal models like sheep and pig are little characterized and still lack the prominent symptoms and neuropathological features. Nonetheless, in accordance to HD patients and R6/2 mouse the porcine HD model displays defects in spermatogenesis. As previously reported on western blot the porcine transgenic TgHD model line bearing a 548aa-120Q N-terminal fragment of human huntingtin express mutant huntingtin in sperm cells. Detailed localization of huntingtin protein epitopes was not determined yet. Ejaculated pig spermatozoa of F1 and F3 generation were used in this study. Indirect immunofluorescence staining of cytospinned spermatozoa was performed using a set of anti- huntingtin specific (EPR5526, MW8) and poly-Q specific (3B5H10) antibodies. In addition mouse R6/2 and human patient spermatozoa were also included to evaluate the relevance of porcine TgHD model. The 3B5H10 immunolabeling results in punctate staining that lines the whole sperm tail of transgenic (Tg) boars. Similar staining pattern is detected using EPR5526 antibody recognizing both mutant and endogenous huntingtin. On contrary to a transgen specific 3B5H10 staining of sperm tail, EPR5526 also detects endogenous huntingtin in wild type (WT) spermatozoa. Total EPR5526 fluorescent signal in TgHD sperm tail is higher than in WT one. The presence of putative aggregated forms of huntingtin cannot be confirmed using MW8 antibody. In summary the endogenous WT huntingtin is a natural constituent of porcine sperm flagella and shows the same localization as mutant protein. Spermatozoa of transgenic animals display elevated levels of huntingtin protein but MW8 positive aggregates cannot be detected.

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P05 Mass spectrometry-based SRM assay for quantification of human mutant huntingtin protein in a transgenic minipig model of Huntington's disease

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Key words: Huntington's disease – huntingtin – selected reaction monitoring

Huntington's disease (HD) is an autosomal dominant hereditary disease caused by expansion of CAG repeats in huntingtin gene (HTT). Translation of this gene results in polyQ stretch in the N-terminus ot the huntingtin protein (Htt). This mutation significantly affects Htt conformation, proteolysis, posttranslational modifications, as well as its ability to bind interacting proteins.

Animal models of HD using genetic manipulations are crucial for testing of the efficacy of novel therapeutic strategies before initiation of clinical trials in human. A transgenic minipig model for HD carrying the first 548 amino acids of human Htt with 124 glutamines has been established at our institute, which has been demonstrated to maintain the germ line transition through several generations. The transgenic animals carry two alleles coding endogenous porcine Htt and one allele for the N-terminal part of human mutant Htt under control of the human promoter.

Protein sequences of human (mutant, transgenic) and porcine (wild type, endogenous) Htt differ in a couple of amino acids which enables to distinguish species-specific forms of Htt and independently quantify mutant and wild type proteins expressed in our model using selected reaction monitoring (SRM).

The first step of this project focused on brain tissue samples has led to the optimization of SRM assays for a set of Htt peptides. To quantify the N-terminal part of the transgenic and endogenous Htt simultaneously, optimal coordinates for all possible tryptic peptides were derived and individual SRM assays were then assembled into a multiplexed fingerprint assay. Development of SRM assays was performed using heavy-labeled, unpurified synthetic version of each peptide. In order to validate SRM assays, peptides containing a heavy-isotope label were spiked into real samples and corresponding transitions were targeted by time-scheduled SRM.

To monitor transgenic and endogenous Htt levels and its lowering in preclinical studies of HD therapy, a sample that could be collected repeatedly in the course of HD progression would be beneficial.

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P06 Mitochondrial impairments in fibroblasts of minipigs transgenic for the N-terminal part of human mutated huntingtin

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Key words: Huntington's disease – large animal model – fibroblast – mitochondria

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by expansion of CAG repeats on chromosome 4p16.3, which results in elongated glutamine tract of huntingtin (Htt). The most pathological effects of HD are focused on the central nervous system but numerous reports had described abnormalities in peripheral tissues. Mutant Htt has been implicated in disruptions of mitochondrial functions whose impairment may contribute to the pathogenesis of HD. The aim of the study was to analyze mitochondrial bioenergetics in cultivated fibroblasts derived from minipigs transgenic for the N-terminal part of human mutated Htt (TgHD).

Cultivated skin fibroblasts from TgHD and WT minipigs of F0–F2 generations (12–60 months) were used for all analyses. Respiratory chain complexes (RCC) activity and amount were analyzed by spectrophotometric, immunoelectrophoretic and immunocapture methods. Respiration was measured by polarography. Mitochondrial ultrastructure, network and reactive oxygen species (ROS) were visualized using fluorescent and electron microscopy. Amount of mtDNA was detected by qPCR.

Pathological changes in mitochondrial ultrastructure (swollen mitochondria, onion like phenotype), network (unequal distribution, isolated segments and fragmentation), increased level of ROS and selectively decreased level of RCC I and IV were detected in TgHD minipig fibroblasts in comparison with WT individuals. Cultured skin fibroblasts could serve as a good tool to investigate mitochondrial impairment as potential disease marker incurred in connection with HD.

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P07 Generation of induced pluripotent stem cells from transgenic minipigs expressing the N-terminal part of the human mutant huntingtin – a new way to study pathogenesis of Huntington's disease

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Key words: Huntington's disease – huntingtin – induced pluripotent stem cells – neural stem cells – cell reprogramming – transgenic minipigs

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by a CAG-triplet expansion in exon 1 of the gene encoding huntingtin (Htt) protein. HD is characterized by motor, cognitive and psychiatric abnormalities resulting from progressive loss of medium spiny neurons in striatum, followed by a cortical and subcorctical atrophy and many other systemic changes. The aim of our research is to study pathogenesis of HD *in vivo* during the lifespan of our pigs as well as *in vitro* using different tissues and cell types to better understand and describe the development of the disease.

Since 2006 the new technology of somatic cells reprogramming to pluripotency has created new opportunities for *in vitro* modelling of various disorders including neurodegenerative diseases such as HD.

We work on generation of induced pluripotent stem cell (iPSCs) lines from our transgenic minipigs. Neural stem cells, porcine embryonic fibroblasts and porcine adult fibroblasts from both transgenic and wild type pigs were isolated and transfected with piggyBac transposon vector

system containing oct-4, klf-4, c-myc and sox-2 reprogramming factors. For transfection itself we used an electroporation-based system from Lonza. Cells were then cultivated in serum-free medium with addition of small molecules such as histone deacetylase inhibitors which can affect the activity of epigenetic regulators and improve reprogramming efficiency. After 1–5 weeks of cultivation we observed morphological changes followed by appearance of colonies morphologically similar to embryonic stem cells. Colonies were then isolated and further cultivated to establish stable cell lines.

Our further research is focused on characterization of iPSCs and their differentiation into neuronal precursors and striatal neurons as well as into other tissues to better understand all differences between transgenic and wild type lines and the role of mutated Htt in the process of embryonic development and cell differentiation.

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P08 Testicular pathology in transgenic minipig boars – in brief

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Key words: transgenic minipig model of Huntington's disease – testes – spermatozoa – reproductive parameters

Huntington's disease (HD) is a neurodegenerative disease in the traditional conception. However, mutated protein huntingtin (mtHtt) is ubiquitously expressed in all tissues of the body. Changes in the peripheral tissues are less-known. Next to the brain, the most abundant expression of huntingtin is in the testes. Accordingly, testicular atrophy was detected in the post mortem samples of HD patients and also in HD transgenic mouse models.

We studied reproductive parameters in the transgenic (TgHD) and wild type (WT) boars of F1 and F2 generations. We evaluated the sperm parameters – sperm count, motility and progressivity. The number of sperms in the ejaculate and their penetration activity were significantly impaired. The motility of the sperms and the results of the survival test of TgHD animals were also lower compare to the WT controls. In contrast, libido sexualis was not decreased. Electron microscopy of sperms of TgHD boars revealed number of the sperm abnormalities like cytoplasmic droplets, deformation of mitochondrial sheath in tail midpiece, folded or coiled tails, instability of acrosome manifested by precocious acrosome reaction. Morphological analysis of the testes by electron microcopy and immunofluorescence showed atrophic seminiferous tubule, loss of gem cells, and high expression of mtHtt. Moreover, we measured the levels of testosterone, FSH, luteinizing hormone, and inhibin alpha in order to eliminate the influence of defects in neurons responsible for hormonal levels. The hormonal screening did not show any differences between WT and TgHD animals.

In conclusion, we show the sperm and testicular pathology in TgHD minipigs caused by the highly abundant expression of mutated huntingtin in testes, and sperms.

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P09 Evidence of amyotrophic lateral sclerosis (ALS) features in SOD1–G93A transgenic swine

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Key words: amyotrophic lateral sclerosis – swine – hSOD1 – phenotypical characterization

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that occurs in two clinically indistinguishable forms: sporadic and familial, the latter mainly linked to mutations in the SOD1 gene. The use of mice carrying the hSOD1G93A mutation is currently widespread in ALS research; however a real improvement of patients prognosis has not yet been obtained (Turner et al., 2013). Another model, more closely related to human species, is strongly demanded by the scientific community that has already foreseen swine as an attractive alternative for modelling human neurodegenerative diseases (Lind et al., 2007). Recently we produced four hSOD1G93A cloned boars (Chieppa et al., 2014), that were bred to establish a transgenic line. To validate the model we performed on the founder animals an extensive molecular and phenotypical characterization.

Copy Number analysis was conducted with the 2–ΔΔct method using the following primers pair: SOD1Fw CATGAACATGGAATCCATGCAGG

and SOD1Rw TAATGGACCAGTGAAGGTGTG for the human SOD1 gene and gapdhFW TGCCACCCAGAAGACTGTGG and gapdhRW ACCTTGCCCACAGCCTTGGC for the glyceraldehyde-3-phosphate dehydrogenase used as reference gene while genomic human DNA was used as a calibrator. Regarding clinical evaluation, motor function and gait dynamics were evaluated using an integrated protocol of digital gait analysis (3D Motion Capture) and surface electromyography (EMG).

The boar #168, carrying 25 to 30 transgene copy number, started to show lameness around twenty seven months of age. At 28 months of age, only a slight incoordination was detectable, which become clear and associated to hypermetria around the 29–30 months of age. Hereinafter appeared dysphagia and regurgitation phenomena meanwhile, during spontaneous walking, the pig kept falling down. Thus the #168 hSOD1G93A transgenic swine showed motor dysfunction and symptoms resembling ALS. The remaining transgenic boars (#174, #204 and #205), carriers of a smaller number of transgene copies, to date are healthy and have reached 48 months of age.

After euthanasia, specific analysis on #168 tissues were performed. Immunohistochemistry and immunofluorescence data revealed granular mutant protein aggregates in the brain and spinal cord, a characteristic ALS hallmark.

All these findings might be consistent with the disease onset, course and end point. However, further molecular and pathological investigations are required to reach a complete and exhaustive characterization of this ALS large animal model.

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P10 Study of protein O-GlcNAcylation in the brain tissue in Huntington's disease

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Key words: Huntington's disease – glycosylation – N-acetylglucosamine – brain tissue

Recent studies suggest a role of O-GlcNacylation – protein modification with N-acetylglucosamine – in the pathophysiology of neurodegenerative diseases. Moreover, altered O-GlcNacylation of mitochondrial proteins has been linked to impaired mitochondrial function. In a Caenorhabditis elegans model of Huntington's disease (HD), genetic manipulation of the O-GlcNAc cycling enzymes OGT and OGA affected proteotoxicity and disease progression. However, no study has been published up until now where the changes of O-GlcNAc signaling in HD patients were analyzed. Our aim was to analyze protein O-GlcNAcylation in the brain tissue of patients with HD.

The analyses were performed in the postmortem brain tissue (Basal Ganglia; BG; Frontal Cortex; FC) of two HD patients (both female, P1: 66 years old, P2: 48 years old) and in corresponding samples from age-matched controls. The global extent of O-GlcNAcylation and levels of O-GlcNAc cycling enzymes, as well as the levels of selected mitochondrial proteins, were analyzed by SDS-PAGE and detected by Western blot. Mitochondrial network was visualized in cultivated dermal fibroblasts (CDF) from P1 using immunofluorescence staining with MitoTracker.

Elevated levels of global protein O-GlcNAcylation were found in BG and FC of P1. Additionaly, increased level of OGT, normalized to tubulin, was detected in BG of both patients. Also in BG of P1 and P2, an unusual OGA pattern was observed with markedly increased ratio of the smaller band to the one with higher molecular weight. Mitochondrial alterations found in the patients included decreased level of multiple pyruvate dehydrogenase subunits (BG), increased level of alpha-ketoglutarate dehydrogenase subunit DLST (BG) and higher fragmentation of the mitochondrial network (CDF).

Our results indicate a disturbed O-GlcNAcylation in the brain tissue of HD patients. We suggest that O-GlcNAcylation dysregulation could be associated with HD pathophysiology, including mitochondrial dysfunction observed in this disease.

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P11 Decreased mitochondrial density and ultrastructural changes of mitochondria in cultivated skin fibroblasts of patients with Huntington's disease

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Key words: Huntington's disease – fibroblasts – mitochondrial ultrastructure – respiratory chain complexes – pyruvate dehydrogenase

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by the expansion of the number of CAG repeats on the gene for protein huntingtin (Htt). More than 36 CAG repeats leads to pathological extension of glutamine tract of Htt which leads to changes of secondary

structure and function of the Htt. Mutant Htt has been implicated in the disruption of multiple cellular processes, including mitochondrial functions whose impairment is emerging as a contributing factor to the pathogenesis of HD. Central nervous system is the most affected in HD but pathologic changes are detectable also in peripheral tissues.

The aim of our study was to analyze the impact of HD on selected bioenergetics' functions, including mitochondrial networks and ultrastructure in cultivated skin fibroblasts. Analyzed group consists of 10 heterozygotic patients with confirmed HD and six healthy adult controls. Number of CAG triplet repeats on the mutated allele ranged between 43 and 53. All fibroblasts were obtained after informed consents. The protein amount of respiratory chain complexes (RCC) was detected by immunoelectrophoretic methods and by dipstick immunocapture analysis (Mitosciences), mitochondrial ultrastructure and network were visualized using fluorescent and transmission electron microscopy and evaluated by Fiji software. Mitochondrial respiration was measured by polarography.

Decreased level of cristae and swollen mitochondria were detected in fibroblasts of all 10 patients. Mitochondrial density was significantly decreased in HD lines in comparison to controls ($p < 0.001$). Protein analysis of patient's fibroblasts showed decreased level of CI subunit NDUFA9 in 8/10 patients and decreased PDH subunit E1-α in 8/10 patients in comparison to controls. Polarographic measurement showed mild decrease in respiration after addition of substrate for complex IV in patient's samples. Our findings of ultrastructural abnormalities in peripheral tissue, such as skin fibroblasts, that are easier to get than autoptic brain, suggests fibroblasts to use as a tool to investigate the pathogenic cascade following huntingtin dysregulation.

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P12 TRACK TgHD minipigs – a Discrimination Test as part of an assessment battery for phenotyping TgHD minipigs

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Key words: animal models – minipig – Huntington's disease – phenotyping – behavioral – preclinical research – cognition – discrimination – reversal learning

The TRACK TgHD minipig study aims to evaluate the potential of the Libechov TgHD minipig model for pre-clinical studies for Huntington's disease. We here introduce the Discrimination Test as part of an assessment battery for minipigs designed to assess the motor, cognitive and behavioral phenotype of a transgenic (Tg) HD minipig model. The Discrimination Test introduced here is thought to primarily target cognitive dysfunction.

The Discrimination Test was performed to assess the feasibility and tolerability of the application in Libechov minipigs and to explore the TgHD minipigs' performance compared with that of wild type (WT) minipigs.

Thirty two female Libechov minipigs – 18 WT and 14 Tg – were available for the study. The TgHD minipigs have with an N-terminal fragment of the huntingtin gene coding for 548aa with 124Q. All animals were housed in six mixed groups (WT and Tg) at the animal facility of the University of Muenster, Germany, in stables with 2 qm per animal enriched with litter and toys.

The Discrimination Test was performed using a special setup. During initial training, the minipigs learnt to leave a startbox (SB A), enter a walkway, open a blue plastic box (bB) as opposed to red and yellow box (rB/yB), and return to SB A. The boxes' position was changed clockwise in subsequent runs. Every box is filled with three cornflakes (reward), but only the bB can be opened. The training was performed twice. During testing 1, preparation time (minipig retained in SB for predefined period of 30 s), initiation time (time required to start run by leaving SB A), exploration time (time required to open correct box) and return time (time required to solve Discrimination task and return to SB A) were recorded and a score was given based on attempts to open and/or investigate bB and/or rB and yB. During testing 2, yB had to be opened instead of bB and yB (reversal learning). Each pig completes trainings (each 5 min), testing 1 (six runs) and testing 2 (six runs) biannually.

The animals show a good tolerance of the method. Cross sectional and longitudinal data analysis is in progress using SPSs 22.0.

The data collected to date shows that implementation of the "discrimination test" is feasible and well tolerated. It is hypothesized that performance of the test is impaired in TgHD minipigs compared to controls. We expect to report between group comparisons at the meeting.

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P13 MRI-based stereotaxic standard brain atlas of the Libechov minipig

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Key words: magnetic resonance imaging – standard brain atlas – Huntington's disease – animal model – transgenic – minipig

In the TRACK TgHD minipig study, we aim to investigate characteristics of the transgenic Libechov minipig as a model for Huntington's disease (HD). Annual magnetic resonance imaging (MRI) scans are performed, including anatomical, spectroscopic and diffusion weighted imaging (DWI) sequences. Quantitative analysis like computing the fractional anisotropy (FA) require a standard brain as target for registration of imaging data. We present here a standard brain template for the Libechov minipig created as target for registration. The standard brain will be used as registration target to allow automated analyses like volumetry, FA analysis and fiber tracking.

In the TRACK TgHD minipig study, we aim to investigate characteristics of the transgenic Libechov minipig as a model for HD. Annual MRI scans are performed, including anatomical, spectroscopic and diffusion weighted imaging (DWI) sequences. Quantitative analysis like computing the fractional anisotropy (FA) require a standard brain as target for registration of imaging data. To date, no standard brain atlas for the Libechov minipig exists.

To provide a stereotaxic standard brain atlas of the Libechov minipig that serves primarily as target for registration of diffusion weighted MR images.

Nine T1-weighted, sagittal acquired MRI volumes of Libechov minipigs were transformed into a common coordinate system and manually skull-stripped. A domestic pig reference atlas (Saikali et al., 2010) was registered on the extracted brains with a landmark and intensity based algorithm (Ghayoor et al., 2013). The BRAINSTools library was used for tissue segmentation (Kim et al., 2014) and structural segmentation (Johnson et al., 2007). The segmentation was cleaned up manually and Multi-atlas joint label fusion (MALF) (Wang and Yushkevich, 2013, Wang et al., 2012) was used to create a standard brain, segmented at regions of interest. The T1-weighted images were acquired with a 3T Philips Achieva scanner.

A total of > 90 T1-weighted 3D brain scans of 32 minipigs have successfully been acquired. A standard brain template could be created as target for registration. The standard brain will be used as registration target to allow automated analyses like volumetry, FA analysis and fiber tracking. The shown images are yet unpublished and likely be released in a Journal of Neuroscience Methods special edition on HD animal models.

Most quantitative analysis methods for MR images require registration of the images to a standard volume. Such templates already exist for several species, but not for the Libechov minipig. The resulting brain template serves as registration target for diffusion weighted MR images and is the first step in creating a Libechov minipig FA template for quantitative analysis of cross sectional and longitudinal DWI data.

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P14 TRACK TgHD minipigs – Tongue Test as a part of an assessment battery for phenotyping TgHD minipigs

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Key words: animal models – minipig – Huntington's disease – phenotyping – behavioral – preclinical research – motor function – tongue

This study aims to assess if transgenic (Tg) minipigs (124 Q) develop symptoms characteristic of human Huntington's disease (HD) in comparison with wild type (WT) minipigs. To investigate this, we developed motor, cognitive and behavioral tests.

We here report a motor function test, the "Tongue Test", and assess the feasibility and tolerability of this assessment.

The WT (n = 18) and Tg (n = 14) minipigs had to perform the same motor function test. The Tongue Test was divided into a training phase and a test phase. During the training phase the pig was presented with a board containing 12 holes measuring the same in diameter and depth. A treat was placed in each hole and had to be picked up with the tongue. A training session lasted until the pig had successfully recovered all 12 treats. It was repeated three times unless a trial lasts more than 5 min, in which case the assessment was stopped. During the test phase the pig was presented with a board that looked the same as the one used in the training session. However, the holes in this test board increased in their depth and the number of successfully recovered treats was counted. As in the training phase the pigs had to perform three runs. Particular determined sections the pigs ran through were counted with a stopwatch. Training and test were videotaped for quality control and further analysis.

The animals show a good tolerance of the method. Cross sectional and longitudinal data analysis is in progress using SPSs 22.0.

TgHD and wild type minipis are capable to perform the Tongue Test successfully and repeatedly. Phenotypical characteristics of the TgHD versus WT comparison will be reported and discussed after completion of further analysis.

Acknowledgement: Funded by the CHDI Foundation.

P15 TRACK TgHD minipigs – Dominance Test as a part of an assessment battery for phenotyping TgHD minipigs

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Key words: animal models – minipig – Huntington's disease – phenotyping – behavioral – preclinical research – dominance

The TRACK TgHD minipig project aims to evaluate the phenotype of the Libechov TgHD minipig model. Established tests for assessing different

domains of possible phenotype manifestation in minipigs are lacking. The test introduced here is thought to primarily target behavioral dysfunction.

We here report a behavioral test, the Dominance Test, and assess the feasibility and tolerability of this assessment.

Thirty two minipigs – 18 wild type (WT) and 14 transgenic (Tg) (124 Q) had to perform the same behavioral test. The animals were housed in six mixed groups (WT and Tg). The Dominance Test was conducted in a narrow aisle divided in half by a removable board. During training, the minipigs learnt to enter the aisle from one side, wait at the door, leave the aisle on the other side and receive a highly favored food treat here. During testing, two animals entered the aisle from either end. The door was opened and the animals faced each other. A "win" was noted for reaching the opposite end of the aisle first, pushing the conspecific aside. All animals per group faced each other once. Number of wins per encounters were compared between WT and Tg minipigs.

The animals show a good tolerance of the method. Cross sectional and longitudinal data analysis is in progress using SPSs 22.0.

TgHD and WT minipigs are able to perform the Dominance Test successfully and repeatedly. Phenotypical characteristics of the TgHD versus WT comparison will be reported and discussed after completion of further analysis.

Acknowledgement: Funded by the CHDI Foundation.

P16 TRACK TgHD minipigs – Hurdle Test as a part of an assessment battery for phenotyping TgHD minipigs

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Key words: animal models – minipig – Huntington's disease – phenotyping – behavioral – preclinical research – motor function – gait

This study aims to assess if transgenic (Tg) minipigs (124 Q) develop symptoms characteristic of human Huntington's disease (HD) in comparison with wild type (WT) minipigs. To investigate this, we developed motor, cognitive and behavioral tests.

We here report a motor function test, the "Hurdle Test", and assess the feasibility and tolerability of this assessment.

The WT (n = 18) and Tg (n = 14) minipigs had to perform the same motor function test. The aim of the Hurdle Test was to assess the gait of the pigs in a more challenging environment than the trot assessed during the GAITRite®. The test has some similarity to the tandem walking sub-item of the UHDRS-TMS performed in humans which also needs higher coordinative skills from the patients than performing the normal gait. It was hypothesized that the Hurdle Test is able to assess deficits in the coordination of the gait in TgHD minipigs earlier, because of the more complex and challenging environment. Furthermore it was supposed to be a good supplement to the GAITRite®.

The test assessed general motor function of the minipigs while walking over hurdles and contained a test phase only, since no specific training is needed. The pigs had to perform six runs while the assessment of each pig was videotaped for independent re-analysis and quality control of assessments and gait patterns.

The animals show a good tolerance of the method. Cross sectional and longitudinal data analysis is in progress using SPSs 22.0.

TgHD and WT minipigs are capable to perform the Hurdle Test successfully and repeatedly. Phenotypical characteristics of the TgHD versus WT comparison will be reported and discussed after completion of further analysis.

Acknowledgement: Funded by the CHDI Foundation.

P17 TRACK TgHD minipigs – a Stress Level Test as a part of an assessment battery for phenotyping TgHD minipigs

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Key words: animal models – minipig – Huntington's disease – phenotyping – behavioral – preclinical research – stress level test – saliva – cortisol

This study aims to evaluate transgenic (Tg) minipigs (124 Q) as a large animal model for pre-clinical studies for Huntington's disease (HD). We here introduce the Stress Level Test as a part of an assessment battery for minipigs designed to assess the motor, cognitive and behavioral phenotype of a transgenic (Tg) HD minipig model. The Stress Level Test introduced here is an assessment to explore behavior. Furthermore the test include a biomarker measurement.

Stress Level Test was performed to explore the feasibility and tolerability of the setup in Libechov minipigs and to study the TgHD minipigs' behavior and biomarkers compared with that of wild type (WT) minipigs.

Thirty two female Libechov minipigs – 18 WT and 14 Tg – were used for this study. The TgHD (124Q) minipigs have with an N-terminal fragment of the huntingtin gene coding for 548aa. All animals were housed in six mixed groups (WT and Tg) at the animal facility of the University of Muenster, Germany, in stables with 2 qm per animal enriched with litter and toys.

Several studies showed a higher cortisol level in HD patients in comparison to control groups. For this reason a stress level test was developed, where cortisol samples are collected standardized before, during and after hoof trimming. The trimming situation in the self-designed

hoof trimming stand was unaccustomed and new for the animals. Additionally a pulse oximeter is fastened on the animal's tail, and heart rate is documented every 30 seconds to detect the stress level. Respiratory rate and body temperature of the animal are measured frequently.

The animals show a good tolerance of the method. Cross sectional and longitudinal data analysis is in progress using SPSs 22.0.

The data collected to date shows that implementation of the Stress Level Test is feasible and well tolerated. It is hypothesized that the behavior and the biomarkers differ between TgHD and WT minipigs. We expect to report between group comparisons at the meeting.

Acknowledgement: Funded by the CHDI Foundation.

P18 Mitochondrial functions in spermatozoa of minipig boars carrying transgene with the N-terminal part of human mutated huntingtin

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Key words: Huntington disease – large animal model – spermatozoa – mitochondria – pig

Huntington's disease (HD), an autosomal dominant neurodegenerative disorder caused by CAG repetition expansion coding glutamine (Q) in sequence of huntingtin gene (HTT; human chromosome 4p16.3), is manifested by central nervous system pathologies and also other abnormalities expressed in peripheral tissues.

Mutant Htt (mtHtt) was described to affect cellular functions due to polyQ-chain cytotoxicity including impairment of mitochondrial metabolism, glycolysis or protein homeostasis.

The aim of this study was to analyse mitochondrial functions in spermatozoa obtained from transgenic minipig boars (TgHD) carrying the N-terminal part of human mtHtt.

Spermatozoa were collected from TgHD and healthy controls from four generations (F0–F3) at the age between 12–60 months. Respiratory chain complexes (RCC) activities and content were analysed by spectrophotometry and imunoelectrophoresis respectively. Oxygen consumption was measured by high-resolution respirometry (Oroboros-2k). Mitochondrial energy generating system (MEGS) capacity was characterized by oxidation rate of radiolabeled substrates.

The presence of human mtHtt in sperm tails significantly disturbed bioenergetics functions of mitochondria in spermatozoa of TgHD boars. Impaired mitochondrial respiration, reduced MEGS capacity and decreased RCC II amount and activity was found in sperm of TgHD boars in comparison with age-related controls. These results support findings from TgHD sperm motility and *in vitro* penetration tests.

Spermatozoa are useful material for non-invasive monitoring of mitochondrial impairment in connection with HD even in presymptomatic stages of disease, but further monitoring of pathologic phenotype development in this large animal model is needed.

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P19 Characterization of immune cell function in N-terminal fragment minipig model of Huntington's disease

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Key words: neuroinflammation – innate immune system – microglia – myeloid cells – cytokines – minipig model of Huntington's disease

Huntington's disease (HD) is an autosomal dominantly inherited neuropsychiatric degenerative, progressive, and fatal condition. Any preventive or disease-modifying therapies are not available so far. Therapeutic interventions can only target symptoms. Whilst the primary pathology in HD is believed to arise from massive degeneration of neurons in the basal ganglia, the expression of mutant huntingtin (mtHtt) has been detected in all examined tissues. Thus the mutation in non-neuronal cells both within the brain and in periphery contributes to the HD pathology. Neuroinflammation, especially microglia activation, is now well characterised feature of neurodegenerative diseases. Immune system disorder outside the central nervous system (CNS) is also increasingly recognised to be involved in the pathogenesis of HD. Given evidence that mutated huntingtin is expressed in peripheral immune cells, it is possible that inflammatory changes detected in peripheral tissues may reflect the inflammatory process in CNS.

Utilising the transgenic porcine HD (TgHD) model bearing N-terminal fragment of human mutant huntingtin and the quantitative proteomic approach (bead-based Luminex xMAP technology), we monitored the immune system dysfunction in HD myeloid cells (including monocytes, macrophages and microglia) and searched for candidate biomarkers of HD onset and progression. Age-matched TgHD and wild type (WT) minipigs with similar genetic background and before the onset of clinical symptoms were used in this study.

Our results provide significant increase in IL-1β and IL-8 levels in non-stimulated microglial cells isolated from TgHD minipigs indicating hyperactivity of these cells. After stimulation, the levels of all measured cytokines were increased in both TgHD and WT animals compared to non-stimulated. However, it was evident that IFNα concentration in responses to stimulation was significantly reduced in TgHD minipigs. Additionally, IL-10 levels were significantly decreased in TgHD minipigs after stimulation. Measurements of cytokine levels in monocyte/macrophage secretomes of non-stimulated cells and

in response to stimulation did not reveal any significant differences between TgHD and WT animals despite the observation that the levels of measured cytokines were increased in both stimulated TgHD and WT animals.

The cytokine alterations reveal involvement of innate immune system as well as possible lack of adaptive immune response in CNS. Using the measurement of cytokine levels, we will continue to monitor the disease progression in pre-manifest stages in transgenic minipigs. Better understanding of the earliest changes in brain and peripheral immune cells could lead to development of preventive or disease-modifying therapies.

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P20 Histological characterization of 36 months old TgHD minipigs

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Key words: Huntington's disease – minipig – immunohistochemistry – brain

The Libechov transgenic minipigs for Huntington's disease (HD) carry the N-terminal part of human mutated huntingtin gene with an expanded CAG repeats coding protein containing 124Q. Even though a lot of rodent models for HD were genetically engineered, the minipigs represent a promising model for the research of HD pathogenesis due to its similarities with human.

In this study, we used three wild type (WT) and three transgenic (TgHD) minipigs of F2 generation (36 months old) and immunohistochemical methods and image analysis techniques were employed for qualitative detection and quantitative determination of protein expression in the brain tissue. Because of mutant huntingtin (mtHtt) affects not only striatum (even if mainly) but also cortex, we evaluated selected cortical areas (motor, somatosensory and insular cortex). In this way we utilized all obtained information from staining coronal section and monitored additional important areas in which processes associated with mtHtt presence got underway. The specificity of the primary antibodies was verified by Western Blot and comparative immunohistochemistry (IHC) of WT and TgHD mouse (R6/2, 12 weeks old) brain sections. The main attention was focused on visualization of mutant huntingtin and its aggregation by the following antibodies: anti-Htt (BML-PW0595 and EPR5526), anti-polyQ (1C2) and anti-aggregated mtHtt (MW8). It is known that mtHtt is expressed in all cell types of the brain and in each of them mtHtt sets off a cascade of events resulting in a selective neuronal loss and an activation of glial cells in the striatum and the cortex. For this purpose, we used anti Iba-1, anti-GFAP and anti-DARPP32 antibodies. Finally, we carried out also histochemical examination with Toluidine blue and Luxol fast blue in coronal brain sections for detection of changes in cellularity and myelinization.

The results of IHC staining revealed increased expression of huntingtin (endogenous and mutant) in TgHD brain. We probably detected the first mtHtt aggregates. The DARPP32 labeling showed the reduced level of expression in striatum of TgHD compared to WT animals. Considering that DARPP32 is a selective marker of striatal medium spiny neurons, our findings suggest a partial loss of inhibitory function of these neurons on dopaminergic signaling in striatum of TgHD minipig brain.

We conclude that our results can contribute to better characterization of the minipig model of HD.

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P21 Czech Huntington Association

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Key words: Huntington's disease in Czech Republic

In 2013, 432 patients were diagnosed with Huntington's disease (HD) in the Czech Republic (CR). Considering the population size in CR, the HD prevalence is very low. Probably this is the main reason, why not only Czech society but also medical doctors are not familiar with HD problematic. HD patients and families lack the most the general understanding and educated personnel in hospitals and residential facilities.

Therefore, Czech Huntington Association (CzHA) was found to support and help HD families. The main goal is improving of quality of life and standards of care for HD families in CR. CzHA aims at psychological support of the people at risk and their protection against discrimination and support for caregivers and children from HD families. CzHA is a member of international Huntington Associations and have one active study centre within the scope of European Huntington's Disease Network.

CzHA faces the problems with the lack of volunteers and professionals, especially child and adolescence psychiatrists and psychologists and also family therapists.

CzHA helped to start with the use of preimplantation genetic diagnosis in CR and managed covering for its costs from public health insurance.

CzHA organizes recondition-educational weekend stays for patients, publish periodical bulletin Archa and brochures about living with HD, cooperates with residential facilities and educates their personnel in specific aspects of caring about HD patients.

Our long-term effort is to raise public awareness about HD.

P22 TRACK TgHD minipig – startbox back and forth test as a part of an assessment battery for phenotyping TgHD minipigs

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Key words: animal models – minipig – Huntington's disease – phenotyping – behavioral – preclinical research – cognition

This study aims to investigate characteristics of the TgHD Libechov minipig as an animal model for Huntington's disease (HD). The "startbox back and forth test" introduced here is part of the assessment battery for phenotyping TgHD minipigs.

The aim of this study is to assess the feasibility and tolerability of applying the "startbox back and forth test" in minipigs and explore its sensitivity to detect cross-sectional and longitudinal differences between transgenic (Tg) HD and wild type (WT) minipigs.

Fourteen TgHD (124 Q) and eighteen WT minipigs were included. The setup the pigs were trained and tested in consisted of a walkway and two startboxes (SB 1 and SB 2) on both ends, connected through trapdoors. In the training phase, minipigs were trained to run back and forth between SB 1 and SB 2 and were rewarded when reaching the opposite startbox. During the testing phase the pigs were not rewarded, while the option to run back and forth was maintained. The learning target in this test was not to run back and forth, because of the missing reward. During training and testing, completed runs within 5 min were counted. The test was stopped when a pig stayed 1 min either in a startbox or in the walkway (assuming that it had learnt there was no reward to expect). Biannually, each pig completed two training sessions and subsequently the testing session was performed.

All data has been successfully acquired in all TgHD and WT minipigs suggesting good tolerance of the method. An ANOVA analysis comparing the differences in behavior between TgHD and WT minipigs is in progress.

The data collected to date demonstrates that application of the "startbox back and forth test" is feasible and well tolerated. It is hypothesized that recognition of the lack of being awarded is impaired in TgHD minipigs compared to controls. This is currently assessed and we expect to report first results at the meeting.

Acknowledgement: Funded by the CHDI Foundation.

P23 TRACK TgHD minipigs – GAITRite® as a part of an assessment battery for phenotyping TgHD minipigs

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Key words: animal models – minipig – Huntington's disease – phenotyping – behavioral – preclinical research – motor function – GAITRite®

Human Huntington's disease (HD) patients show motor disabilities such as decreased stride length and impaired dynamic balance. The GAITRite® system is part of the study to detect phenotypical changes in transgenic (Tg) HD Libechov minipigs compared to wild type (WT) Libechov minipigs.

The aim of this study is to detect gait impairment in TgHD minipigs longitudinally, compared to WT minipigs. The feasibility and applicability of the GAITRite® System is tested.

The project included fourteen TgHD (124 Q) and eighteen WT minipigs. The pigs were trained and tested on the Platinum® 2006 MAPCIR GAITRite®, the computer system used for quadruped gait is the GAITFour® software. While the pigs walked over the carpet, footprints were automatically identified and recorded in the software. All sessions are recorded by video.

Before the training session, pigs were conditioned to a clicker. During the trainings, pigs were taught by clicker to trot in an even speed over the carpet. As soon as the pigs learnt how to perform the GAITRite®, no clicker was used anymore. Cornflakes were given for motivation as a treat. The pigs had to perform 15 trots in an even speed.

The GAITRite® was performed biannually by each pig.

All data has been successfully acquired in all TgHD and WT minipigs. As data is collected longitudinally, analysis is in progress.

The GAITRite® System is feasible and well tolerated by the minipigs. The data collection is to be analyzed. It is hypothesized, that TgHD minipigs will show gait impairment, compared to WT minipigs and a deterioration compared to themselves, longitudinally.

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P24 Targeted mass spectrometry based assay for monitoring neuronal differentiation

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Key words: pluripotent cells – neural differentiation – neurons – mass spectrometry – selected reaction monitoring

Embryonic stem cells (ESCs) are self-renewing pluripotent cells that have the capability of differentiating into a wide variety of cell types. ESC-derived neural precursors undergo currently preclinical investigation for testing their potential in cell-based therapies of neurological disorders due to their successful expansion and differentiation *in vitro* giving rise to

neurons, astrocytes and oligodendrocytes. Despite extensive research focused on the lineage-specific differentiation of neural stem cells (NSCs), much remains to be elucidated before their translation toward clinical applications. In order to efficiently generate a homogeneous population of neural precursors required for transplantation with their anticipated *in vivo* differentiation and simultaneous elimination of teratoma formation, it is necessary to fully characterize the developmental stages of neural cells at molecular level.

Using targeted mass spectrometry approach, based on selected reaction monitoring (SRM), our study aimed at the characterization of human NSCs upon neuronal differentiation induced by BDNF (Brain Derived Neurotrophic Factor) and GDNF (Glial Derived Neurotrophic Factor). Instead of currently used immunocytochemistry, SRM was applied to generate quantitative information on typical protein markers of neuronal differentiation. Doublecortin, Tuj1 and MAP2 were found gradually rising during NSC differentiation into mature neurons. To evaluate the presence of supporting glial cells as well as possible residual pluripotent cells, the levels of other relevant protein markers were also measured by SRM.

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P25 The effect of melatonin on proliferation of primary porcine cells expressing mutated huntingtin

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Key words: Huntington's disease – melatonin – time lapse microscopy – minipig model – proliferation curves – skin fibroblasts

According to the recent studies, melatonin might play an important role in Huntington's disease (HD) and act as a novel therapeutic approach in the treatment of the disease. HD, the inherited neurodegenerative disorder, is accompanied by gradual melatonin reduction as it progresses.

Melatonin in normal cells (non-tumor) has the anti-apoptotic ability due to its antioxidant property and its ability to prevent the activation of p53. Furthermore, melatonin increases the expression of BDNF (brain derived neurotrophic factor) and other neuroprotective factors.

The aim of this study was to evaluate the nontoxic dose of melatonin for primary skin fibroblasts isolated from minipigs transgenic for the N-terminal part of human mutated huntingtin (TgHD), and the effect of melatonin treatment to these cells exposed to genotoxic stress.

Cells were cultured in medium supplemented with different doses of melatonin. Using time lapse microscopy, we estimated the effect of decreasing melatonin concentrations by analyzing the proliferation curves.

We show that higher doses of melatonin are toxic for primary porcine fibroblasts. Interestingly, TgHD cells were more sensitive to these doses of melatonin treatment than wild type cells. We evaluated the effective dose of melatonin and demonstrated its rescue proliferative effect on porcine primary cells exposed to genotoxic stress.

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