

CONFERENCE PROGRAM

MONDAY FEBRUARY 22ND, 2016		
12:00pm–5:00pm	Registration & Poster Setup	Ballroom Foyer
	AN UPDATE FROM CHDI Chaired by: Robert Pacifici, PhD, <i>CHDI</i>	Ballroom
2:00pm–2:30pm	Preclinical Activities: Biology Thomas F. Vogt, PhD, <i>CHDI</i>	Ballroom
2:30pm–3:00pm	Preclinical Activities: Chemistry Celia Dominguez, PhD, <i>CHDI</i>	Ballroom
3:00pm–3:15pm	Preclinical Q&A	Ballroom
3:15pm–4:15pm	Clinical Activities Cristina Sampaio, MD, PhD, <i>CHDI</i>	Ballroom
4:15pm–4:30pm	Clinical Q&A	Ballroom
4:30pm–5:00pm	Break	Ballroom Foyer
5:00pm–5:30pm	Welcome Robert Pacifici, PhD, <i>CHDI</i>	Ballroom
5:30pm–7:00pm	Keynote Speakers Astri Arnesen, <i>European Huntington Association</i> Svein Olaf Olsen, <i>European Huntington Association</i>	Ballroom
7:00pm–10:00pm	Buffet Dinner Reception	Fire Pit & Croquet Lawn
TUESDAY FEBRUARY 23RD, 2016		
7:00am–8:00am	Yoga	PSYC/The Spa
7:30am–9:00am	Buffet Breakfast	Fire Pit & Croquet Lawn
SESSION I	HUNTINGTIN: STRUCTURAL INSIGHTS LEADING TO THERAPEUTIC APPROACHES Chaired by: Liz Doherty, PhD, <i>CHDI</i> Elena Cattaneo, MD, <i>University of Milano</i>	Ballroom
9:00am–9:10am	Introduction Elena Cattaneo, MD, <i>University of Milano</i>	Ballroom
9:10am–9:45am	Effect of polyQ expansions on the solution structure and misfolding of huntingtin Ralf Langen, PhD, <i>University of Southern California</i>	Ballroom
9:45am–10:20am	Elucidating the sequence and structural determinants of huntingtin aggregation and toxicity using protein semisynthesis and single molecule techniques Hilal A. Lashuel, PhD, <i>Ecole Polytechnique Fédérale de Lausanne</i>	Ballroom
10:20am–10:35am	Break	Ballroom Foyer
10:35am–11:10am	A structure/function analysis in knock-in mice of the huntingtin N-terminal domains encoded by exon 1 Scott Zeitlin, PhD, <i>University of Virginia</i>	Ballroom
11:10am–11:45am	Huntingtin and the control of intracellular dynamics: Role of proteolysis and non-polyQ fragments Frédéric Saudou, PhD, <i>Grenoble Institute of Neurosciences</i>	Ballroom
11:45am–12:20pm	Biochemical-genetic investigation of the influence of the polyglutamine tract on huntingtin structure and function Ihn Sik Seong, PhD, <i>Massachusetts General Hospital</i>	Ballroom
12:20pm–2:00pm	Buffet Lunch	Fire Pit & Croquet Lawn

PROGRAM

SESSION II	HUNTINGTIN GENE AND GENOME: EVER CHANGING Chaired by: Hilary Wilkinson, PhD, <i>CHDI</i> Arnold Levine, PhD, <i>Institute for Advanced Study</i>	Ballroom
2:00pm–2:05pm	Introduction Hilary Wilkinson, PhD, <i>CHDI</i>	Ballroom
2:05pm–2:40pm	DNA methylation age analysis of human Huntington’s disease Steve Horvath, PhD, ScD, <i>University of California, Los Angeles</i>	Ballroom
2:40pm–3:15pm	Striatal super-enhancer signature is altered in Huntington’s disease: Enhancer regions, a therapeutic target? Karine Merienne, PhD, <i>University of Strasbourg</i>	Ballroom
3:15pm–3:50pm	Instability of the Huntington’s disease CAG repeat mutation: Genetic approaches towards dissecting mechanism and the relationship to pathogenesis Vanessa Wheeler, PhD, <i>Massachusetts General Hospital/Harvard Medical School</i>	Ballroom
3:50pm–4:05pm	Break	Ballroom Foyer
4:05pm–4:40pm	RAN translation in Huntington’s disease Laura P.W. Ranum, PhD, <i>University of Florida</i>	Ballroom
4:40pm–5:15pm	Huntingtin functions in response to oxidative DNA damage via a novel signaling pathway that co-regulates huntingtin and P53 activity in ROS DNA-damaged cells Ray Truant, PhD, <i>McMaster University</i>	Ballroom
5:15pm–5:20pm	Closing Summary Arnold Levine, PhD, <i>Institute for Advanced Study</i>	Ballroom
<p><i>We will not host a dinner reception this evening. Downtown Palm Springs has many wonderful restaurants. Transportation to and from downtown will be provided. Check with the registration desk for restaurant recommendations, shuttle schedule and pick-up/ drop-off locations. If you prefer to stay on campus, Norma’s at Night and Mister Parker’s will be open for business.</i></p>		
WEDNESDAY FEBRUARY 24TH, 2016		
7:00am–8:00am	Yoga	PSYC/The Spa
7:30am–9:00am	Buffet Breakfast	Fire Pit & Croquet Lawn
SESSION III	TARGETED THERAPIES FOR HUNTINGTIN LOWERING Chaired by: Mark Rose, PhD, <i>CHDI</i> Benhur Lee, MD, <i>Mount Sinai Hospital</i>	Ballroom
9:00am–9:05am	Introduction Benhur Lee, MD, <i>Mount Sinai Hospital</i>	Ballroom
9:05am–9:30am	Beacons along the way – Biomarkers to monitor HTT lowering in the brain Ignacio Munoz-Sanjuan, PhD, <i>CHDI</i>	Ballroom
9:30am–10:00am	Refining experimental gene therapies for Huntington’s and other diseases Pavlina Konstantinova, PhD, <i>uniQure</i>	Ballroom
10:00am–10:30am	Caveats and challenges in gene silencing for Huntington’s disease: Lessons from mice and monkeys Jodi McBride, PhD, <i>Oregon Health and Science University</i>	Ballroom
10:30am–10:45am	Break	Ballroom Foyer
10:45am–11:15am	Development and applications of genome editing technologies Feng Zhang, PhD, <i>The Broad Institute of MIT and Harvard</i> (live remote presentation with Q&A)	Ballroom

11:15am-11:45am	Autophagy and other pathways that protect against neurodegeneration David Rubinsztein, PhD, <i>University of Cambridge</i>	Ballroom
11:45am-12:15pm	Selective clearance of mHtt through exploitation of the ubiquitin-proteasome pathway Eric Reits, PhD, <i>University of Amsterdam</i>	Ballroom
12:15pm-12:20pm	Closing Summary Mark Rose, PhD, <i>CHDI</i>	Ballroom
12:20pm-2:00pm	Buffet Lunch	Fire Pit & Croquet Lawn
SESSION IV	FORMAL POSTER PRESENTATIONS Chaired by: Richard Chen, PhD, <i>CHDI</i> Poster Judges: Claudia Hung, PhD, <i>McMaster University</i> , 2015 First Prize Winner Andrea Caricasole, PhD, <i>IRBM Science Park</i> Mahmoud Pouladi, PhD, <i>National University of Singapore</i>	Ballroom Lawn & Palm Court
1:00pm-2:15pm	GROUP A • Huntingtin: Function/Lowering/Structure • HD Biological Mechanisms	Ballroom Lawn & Palm Court
2:15pm-3:30pm	GROUP B • HD Models • Systems Biology • Translational Medicine	Ballroom Lawn & Palm Court
	CLINICAL TRIALS UPDATE BLITZ Chaired by: Andrew Wood, PhD, <i>CHDI</i>	Ballroom
3:30pm-3:45pm	SRX246 Trial Neal G. Simon, PhD, <i>Azevan Pharmaceuticals</i>	Ballroom
3:45pm-4:00pm	PDE10A ICM Trial Marielle Delnomdedieu, PhD, <i>Pfizer</i>	Ballroom
4:00pm-4:15pm	SIGNAL Trial Maurice Zauderer, PhD, <i>Vaccinex</i>	Ballroom
4:15pm-4:30pm	HTT Rx CS1 Trial Sarah Tabrizi, PhD, FRCP, <i>University College London (for Ionis Pharmaceuticals)</i>	Ballroom
	FEATURED SPEAKER	Ballroom
4:30pm-4:35pm	Introduction Jen Ware, PhD, <i>CHDI</i>	Ballroom
4:35pm-5:30pm	Reproducibility: Crisis or opportunity? Marcus Munafò, PhD, <i>University of Bristol</i>	Ballroom
5:30pm-6:15pm	Top 3 Poster Presentations	Ballroom
6:15pm-7:00pm	Cocktail Reception with Poster Voting	Ballroom Lawn & Palm Court
7:00pm-10:00pm	Buffet Dinner Reception	The Living Room

PROGRAM

THURSDAY FEBRUARY 25TH, 2016		
7:00am–8:00am	Yoga	PSYC/The Spa
7:30am–9:00am	Buffet Breakfast	Fire Pit & Croquet Lawn
SESSION V	REPLACING CELLS – RESTORING NETWORKS Chaired by: Dan Felsenfeld, PhD, <i>CHDI</i> Lee Rubin, PhD, <i>Harvard University</i>	Ballroom
9:00am–9:10am	Introduction Lee Rubin, PhD, <i>Harvard University</i>	Ballroom
9:10am–9:45am	Phenotypic signature of HD in isogenic human embryonic stem cells Ali Brivanlou, PhD, <i>The Rockefeller University</i>	Ballroom
9:45am–10:20am	A role of the protein kinase JNK3 on mutant huntingtin–induced neuropathology Gerardo Morfini, PhD, <i>University of Illinois at Chicago</i>	Ballroom
10:20am–10:55am	Reprogramming neurons and circuits in vivo Paola Arlotta, PhD, <i>Harvard University</i>	Ballroom
10:55am–11:10am	Break	Ballroom Foyer
11:10am–11:45am	Analyses of mood–related neural circuits in relation to Huntington’s disease Ann Graybiel, PhD, <i>Massachusetts Institute of Technology</i>	Ballroom
11:45am–12:20pm	Glia as therapeutic reagents in HD Steve Goldman, MD, PhD, <i>University of Rochester</i>	Ballroom
12:20pm–2:00pm	Buffet Lunch	Fire Pit & Croquet Lawn
SESSION VI	CLINICAL MEASUREMENTS AND MODELING Chaired by: Amrita Mohan, PhD, <i>CHDI</i> Werner Poewe, MD, <i>Medical University Innsbruck</i>	Ballroom
2:00pm–2:10pm	Introduction Amrita Mohan, PhD, <i>CHDI</i>	Ballroom
2:10pm–2:50pm	Huntington’s disease measurements and models: Quality and relevance Douglas R. Langbehn, MD, PhD, <i>University of Iowa</i>	Ballroom
2:50pm–3:30pm	Clinical rating scales in Huntington’s disease: Validation, validation, validation Tiago A. Mestre, MD, MSc, <i>University of Ottawa</i>	Ballroom
3:30pm–3:50pm	Break	Ballroom Foyer
3:50pm–4:30pm	Validation of a prognostic index for Huntington’s disease Jeffrey D. Long, PhD, <i>University of Iowa</i>	Ballroom
4:30pm–5:10pm	Understanding Huntington’s disease progression: A multi–level probabilistic modeling approach Jianying Hu, PhD, <i>IBM T.J. Watson Research Center</i>	Ballroom
5:10pm–5:20pm	Closing Summary Werner Poewe, MD, <i>Medical University Innsbruck</i>	Ballroom
5:20pm–6:20pm	Poster Competition Awards Charles Sabine, <i>NBC News Correspondent</i>	Ballroom
	Closing Remarks Robert Pacifici, PhD, <i>CHDI</i>	Ballroom
7:30pm–8:30pm	Cocktail Reception	Gene Autry House
8:30pm–10:30pm	Farewell Dinner Banquet	Gene Autry Lawn

SPEAKER ABSTRACTS

SESSION I - HUNTINGTIN: STRUCTURAL INSIGHTS LEADING TO THERAPEUTIC APPROACHES

Effect of polyQ expansions on the solution structure and misfolding of huntingtin

Ralf Langen, PhD

University of Southern California

Huntington's disease is caused by polyQ expansions in the huntingtin (htt) protein. While the mechanisms of toxicity remain under investigation, it is likely that the polyQ expansions alter huntingtin structure and cause the formation of toxic states. Such toxic states could include monomeric protein with altered functional properties or oligomeric/fibrillar misfolded forms of htt. Restoring the conformational properties of normal htt may therefore be a potential therapeutic avenue. Proof-of-principle for this notion comes from transthyretin-related hereditary amyloidosis (also called familial amyloid neuropathy, FAP), where a small molecule drug, tafamidis, maintains the native fold and prevents the conversion into toxic misfolded forms. Structural information was essential for the design of tafamidis and will likely be essential for applying analogous approaches to HD.

Structural studies on htt or its exon1 (httex1) with polyQ expansions are complicated by the aggregation prone nature of the proteins and technical difficulties of many high-resolution structural methods. We therefore first developed methods for preparing stable monomeric, oligomeric or fibrillar species of httex1 and investigated their structures using a combination of biophysical methods including EPR, solution NMR, solid-state NMR, circular dichroism, fluorescence and electron microscopy. We found that monomeric httex1 is largely unfolded, but that it has residual α -helical structure in its N-terminal region and strong polyproline II helical structure in its C-terminal region. Interestingly, the α -helical structure increased with increasing polyQ length leading to increased rigidity that effectively freezes out the motion of httex1. This polyQ-dependent enhancement of rigidity could contribute to the toxicity by altering the functional or protein-protein interaction properties of httex1. By monitoring misfolding over time, we found that httex1 misfolding is a highly seeded process. While monomeric httex1 with expanded Q-lengths was highly stable for many hours even at tens of micromolar concentrations, the addition of seeds significantly sped up aggregation. Monitoring this seeded reaction has provided us with insights regarding the mechanism of misfolding and allowed us to investigate molecules that promote or inhibit misfolding. Finally, we have generated different types of misfolded httex1 fibrils and oligomers. These forms include oligomers and fibrils previously suggested to be toxic or only weakly toxic. In all cases, the central β -sheet core region of the misfolded species was located in the polyQ region surrounded by extended polyproline II helix bristles. The central difference among the structures was that these bristles are solvent exposed in the putatively toxic structures while they are bundled up in the less toxic forms. These data suggest the possibility that interaction with the polyproline II bristles may additionally contribute to pathogenesis.

Tuesday February 23rd, 2016
9:10am – 9:45am

SESSION I - HUNTINGTIN: STRUCTURAL INSIGHTS LEADING TO THERAPEUTIC APPROACHES**Elucidating the sequence and structural determinants of huntingtin aggregation and toxicity using protein semisynthesis and single molecule techniques****Hilal A. Lashuel, PhD**

Ecole Polytechnique Fédérale de Lausanne

Increasing evidence suggest that post-translational modifications (PTMs), such as phosphorylation, ubiquitination and SUMOylation, within the N-terminal 17 residues of the huntingtin protein play important roles in modulating the aggregation, subcellular localization and toxicity of full-length and N-terminal huntingtin protein fragments (Htt) in cellular and animal models of Huntington's disease. However, the lack of knowledge about the natural enzymes involved in regulating these PTMs has hampered studies aimed at elucidating the molecular mechanisms by which these modifications influence the function of Htt in health and disease. To address this knowledge gap, our group has developed and optimized several protein expression and protein semisynthetic strategies and protocols that enable 1) the efficient and rapid production of recombinant expression of tag-free exon1 proteins with polyQ repeat lengths ranging from 6-49Q; 2) site-specific introduction of single or multiple PTMs within the Nt17 region of exon1. In collaboration with the chemistry group at IRBM and Evotec, we have further optimized these protocols to scale up the production of these proteins.

With these highly pure and homogeneous preparations of unmodified and modified forms of exon1 in hand, we applied interdisciplinary approaches that combine standard and advanced biophysical techniques, including circular dichroism (CD), transmission electron microscopy (TEM), atomic force microscopy (AFM), nano-IR spectroscopy and single molecule FRET (In collaboration with Edward Lemke, EMBL) to investigate the effect of polyQ repeat length, the Nt17 domain and its PTMs in regulating the structure of monomeric and aggregated forms of exon1 at the single molecule and nanometric scale. Together, these advances allowed, for the first time, dissection of the role of individual PTMs, including phosphorylation (at T3, S13 and S16), acetylation (K9), ubiquitination and SUMOylation (K6, K9, and K15), in regulating the structure and aggregation of exon1 in vitro and led to the identification of novel PTM-dependent structural switch elements involving cross-talk between different PTMs. These findings and their implication in understanding the sequence and structural determinants of Htt aggregation, toxicity and function in health and disease will be presented and discussed. Finally, preliminary studies conducted in collaboration with IRBM (Andrea Caricasole) demonstrate the great potential of using these highly pure and homogeneous preparations of exon1 as powerful tools to facilitate the development of novel sensitive assays for the detection and quantification of modified forms of Htt and to discover enzymes involved in regulating Htt phosphorylation.

Tuesday February 23rd, 2016

9:45am - 10:20am



SESSION I - HUNTINGTIN: STRUCTURAL INSIGHTS LEADING TO THERAPEUTIC APPROACHES**A structure/function analysis in knock-in mice of the huntingtin N-terminal domains encoded by exon 1****Scott Zeitlin, PhD**

University of Virginia

To understand the role of the huntingtin (Htt) N-terminal domains in normal Htt function and in modulating HD mouse model pathogenesis, we have generated and are characterizing knock-in mouse models that express versions of normal Htt lacking the N17 domain (Δ N17), the polyQ stretch (Δ Q), the proline-rich region-or PRR (Δ P), a combination of both the polyQ and PRR domains (Δ QP), and all three N-terminal domains encoded by Htt exon 1 (Δ NQP). In homozygosity, all the deletion mutants are born at normal Mendelian frequency, suggesting that the exon-1 encoded domains of Htt are not required for normal embryonic development. As adults, the homozygous deletion mutants exhibit only subtle behavioral phenotypes, and lack obvious neuropathology. However, when the Htt N-terminal domain deletion mutants are expressed together with the CAG140 allele, the individual domain deletions have differential effects on HD mouse model phenotypes. While the Q deletion can rescue some aspects of pathogenesis, the N17 and QP deletions exacerbate some CAG140 phenotypes. To better understand the effect of these deletions on CAG140 pathogenesis, we are in the process of characterizing autophagy in primary striatal and cortical neuronal cultures obtained from postnatal day 5 Htt140Q/ Δ N17, Htt140Q/ Δ QP, wild-type, Htt140Q/ Δ Q and Htt140Q/+ pups. Preliminary results suggest that while autophagic flux is enhanced in the Htt140Q/ Δ Q cultures in comparison to controls, Htt140Q/ Δ N17 cultures exhibit a significant deficit in autophagy.

We are also studying the effect of deleting the Htt PRR in cis to the expanded polyQ stretch in Htt140Q Δ P/+ mice, and the effect of replacing the murine PRR with the human PRR in the normal allele of Htt140Q/7QhuP mice. Consistent with the results obtained from analyzing Htt PRR deletions in other model systems, deletion of the PRR in cis to the 140Q stretch affects mutant Htt aggregation. Our preliminary data indicate that substituting a human PRR for the murine PRR in the normal allele of Htt140Q/7QhuP mice exacerbates HD model phenotypes in comparison to Htt140Q/+ controls. These results suggest that the Htt PRR can modulate the toxic effects of 140Q-Htt expression both in cis and in trans.

Tuesday February 23rd, 2016
10:35am - 11:10am

SESSION I - HUNTINGTIN: STRUCTURAL INSIGHTS LEADING TO THERAPEUTIC APPROACHES

Huntingtin and the control of intracellular dynamics: Role of proteolysis and non-polyQ fragments

Frédéric Saudou, PhD

Grenoble Institute of Neurosciences

Huntington's disease is caused by the abnormal polyglutamine expansion in the N-ter part of huntingtin (HTT), a large protein of 350kDa. Over the past years, we proposed that HTT acts a scaffold for the molecular motors and, through this function, regulates the efficiency and directionality of vesicular transport along microtubules in neurons. This function is conserved in *Drosophila*. In particular, HTT controls the microtubule-based fast axonal transport (FAT) of neurotrophic factors such as BDNF. PolyQ expansion in HTT alters this function, leading to a decrease in neurotrophic support and death of striatal neurons. Interestingly, the defect in transport might not be restricted to axons but could also involve defects in the retrograde transport of TrkB in striatal dendrites.

In addition to the role of HTT in scaffolding the molecular motors both in cortical and striatal neurons, we found that HTT scaffolds GAPDH on vesicles and that vesicular GAPDH is necessary to propel vesicles in GAPDH deficient neurons. Here we will extend these findings and discuss how HTT, by specifically localizing the glycolytic machinery on vesicles, may supply constant energy for the transport of vesicles over long distances in axons. We will also discuss how this machinery is altered in disease.

Finally, we will extend the function of HTT as a scaffold for dynamin1 for regulating intracellular dynamics in health and disease. Indeed, we found, using time- and site-specific control of HTT proteolysis, that specific cleavages are required to disrupt intramolecular interactions within HTT and to cause toxicity in cells and flies. Surprisingly, in addition to the canonical pathogenic N-ter fragments, the C-ter fragments generated, that do not contain the polyQ stretch, induced toxicity via dilation of the endoplasmic reticulum (ER). C-ter HTT bound to dynamin 1 and subsequently impaired its activity at ER membranes. Our findings thus identify a new pathogenic mechanism in HD and suggest that non-polyQ fragments of HTT generated by proteolysis contribute to disease by altering ER dynamics and homeostasis.

Tuesday February 23rd, 2016
11:10am - 11:45am



SESSION I - HUNTINGTIN: STRUCTURAL INSIGHTS LEADING TO THERAPEUTIC APPROACHES**Biochemical-genetic investigation of the influence of the polyglutamine tract on huntingtin structure and function****Ihn Sik Seong, PhD**

Massachusetts General Hospital

The CAG trinucleotide repeat expansion mutation that is the genetic cause of Huntington's disease (HD) elongates a normally variable segment of polyglutamine located sixteen amino acids from the start methionine of the 350kD huntingtin protein to more than about forty residues. To understand this mutation at the level of the full-length huntingtin HEAT/HEAT-like domain molecule itself, we are using a classical biochemical-genetic strategy that couples the physical analyses of a series of highly purified full-length huntingtins with different polyglutamine tracts to the functional activity of these proteins in a validated cell-free assay (Seong et al. HMG (2010) 19:573-583): enhancement of polycomb repressive complex 2-dependent nucleosome deposition of the histone H3K27me3 mark (PRC2-assay). Our results reveal that full-length huntingtin, which is bisected by a protease sensitive hinge site at residues 1184-1254 into two large arms, assumes a spherical α -helical solenoid shape with a large internal cavity, that involves short-, medium- and long-range intramolecular contacts, with the latter mediating the interaction of the amino terminal arm with the carboxyl terminal arm. This 3D shape is globally similar regardless of polyglutamine size. However, polyglutamine length does alter the pattern of full-length huntingtin intramolecular contacts and the pattern of posttranslational modifications, including 'crosstalk' between distant phosphosites, such that huntingtin's polyglutamine tract size and phosphorylation status is associated with the protein's function activity. The 3D closed α -helical shape assumed by full-length huntingtin's HEAT/HEAT-like rich domains, therefore, provides an elegant solution to the conundrum of full-length huntingtin as the polyglutamine tract size dependent disease-initiator. Our results pave the way for high-resolution studies that will be required to determine in detail the influence of its polyglutamine segment on the structure of full-length huntingtin, while our biochemical-genetic approach provides a route to identify factors that can selectively manipulate full-length mutant huntingtin without affecting normal full-length huntingtin activity.

Tuesday February 23rd, 2016

11:45am - 12:20pm

SESSION II - HUNTINGTIN GENE AND GENOME: EVER CHANGING

DNA methylation age analysis of human Huntington's disease

Steve Horvath, PhD, ScD

University of California, Los Angeles

Biological tissue age seems to play an important role in Huntington's disease (HD), e.g. the age of disease onset is strongly related to the number of CAG trinucleotide repeats in the HD gene. It is a plausible hypothesis that HD is associated with an increased biological age of affected tissues, but molecular evidence for this hypothesis has been sparse.

Here, we utilize a quantitative molecular marker of aging (known as the epigenetic clock) to analyze the tissue age of multiple postmortem brain samples from 26 HD patients and 39 controls. The epigenetic clock is a DNA methylation based biomarker of aging that can be used to measure the DNA methylation (DNAm) age of any human tissue, cell type, or fluid that contains DNA with the exception of sperm. Recent results demonstrate that DNAm age captures aspects of organismal age and the biological age of brain tissue. Here we demonstrate that HD is associated with a significant increase in the epigenetic age of specific brain regions (on average 3.3 years) and that the observed age acceleration effect is independent of underlying changes in cell composition.

Our systems biologic analysis of the brain methylation data identified 9 co-methylation modules with a significant association with HD status in several brain regions. Overall, we demonstrate that HD is associated with an increased epigenetic age of brain tissue and more broadly with substantial changes in brain methylation levels.

Tuesday February 23rd, 2016
2:05pm - 2:40pm



SESSION II - HUNTINGTIN GENE AND GENOME: EVER CHANGING

Striatal super-enhancer signature is altered in Huntington's disease: Enhancer regions, a therapeutic target?**Karine Merienne, PhD**

University of Strasbourg

Epigenetic alterations are documented in several models of Huntington's disease (HD). However, whether epigenetic modifications play a causal role in the mechanism leading to transcriptional dysregulation and contribute to disease pathogenesis is unclear. Using the striatum of HD R6/1 mice, we generated RNA-seq and ChIP-seq data, focusing on RNA polymerase II (RNAPII) and histone modifications associated with enhancers, such as H3K27 acetylation (H3K27ac). Integrated analysis of RNA-seq and ChIP-seq data revealed that down-regulated genes in HD mouse striatum display a super-enhancer signature. Super-enhancers are a class of broad enhancers, regulating genes that define cell-type-specific identity and function (e.g. neuronal identity and function genes in neurons). In addition, we show that this super-enhancer signature is altered in HD mouse striatum, since H3K27ac levels and enhancer RNA (eRNA) transcription are selectively decreased at HD striatal super-enhancers, and this correlates with reduced levels of RNAPII at super-enhancer-regulated genes. Finally, H3K27ac ChIP-seq data generated from the striatum of HD patients and control individuals indicate that down-regulated genes associate with broad enhancer regions and H3K27ac signal is decreased at those regions, suggesting that striatal super-enhancer signature is also altered in HD patients. Thus, our data support a mechanism where impaired super-enhancer activity in HD striatum contributes to altered expression of neuronal identity and function genes, defining a class of genes essential to regulation of neuronal activity such as synaptic plasticity and adaptive behavior. We suggest that targeting enhancer activity may be of therapeutic interest.

Tuesday February 23rd, 2016
2:40pm - 3:15pm

SESSION II - HUNTINGTIN GENE AND GENOME: EVER CHANGING

Instability of the Huntington's disease CAG repeat mutation: Genetic approaches towards dissecting mechanism and the relationship to pathogenesis**Vanessa Wheeler, PhD**

Massachusetts General Hospital/Harvard Medical School

The expanded CAG repeat in the Huntington's disease gene, HTT, is the major contributor to disease onset and severity. The HTT CAG repeat expands somatically in a time-dependent and cell-type/tissue-dependent manner, with medium-spiny neurons of the striatum exhibiting particularly dramatic expansions. We have shown in genetic studies in HttQ111 knock-in mice that genes in the mismatch repair (MMR) pathway are absolutely required for somatic CAG expansion and that the same genes enhance the pathogenic process. The relevance of these findings to human disease is highlighted by a recent genome-wide association study that identified MMR gene MLH1 as a candidate modifier of disease onset and MMR as a disease onset-associated pathway. While these findings are consistent with somatic expansion as a disease modifier, they do not rule out an alternative hypothesis that MLH1 and other MMR genes modify disease pathogenesis via mechanisms unrelated to the instability of the CAG repeat. Understanding the pathways by which MMR genes modify pathogenesis has important implications for the development of disease-modifying therapies.

To better understand the role of somatic expansion and MMR genes as disease modifiers we are performing genetic studies in HD patients and in mice. In mice, we are utilizing novel Htt CAG knock-in lines that carry either a pure CAG repeat or a CAG repeat tract that is interrupted with CAA residues, with pairs of pure and interrupted repeat-containing mice expressing huntingtin with approximately matching glutamine tract lengths (45, 80, 105). The pure CAG knock-in mice exhibit somatic expansion that increases with mouse age and constitutive CAG repeat length and occurs in a tissue-specific manner. In contrast, the interrupted repeat configuration results in complete repeat stabilization. These models thus allow us to investigate the potential impact of somatic CAG expansion on disease expression and provide tools to test whether genetic modifiers such as MLH1 impact HD pathogenesis via mechanisms that are dependent on or independent of somatic expansion. Molecular, histological and behavioral phenotyping of the pure and interrupted mice is ongoing and results from these analyses will be presented.

Tuesday February 23rd, 2016
3:15pm - 3:50pm



SESSION II - HUNTINGTIN GENE AND GENOME: EVER CHANGING**RAN translation in Huntington's disease****Laura P.W. Ranum, PhD**

University of Florida

Huntington disease (HD) is caused by a CAG•CTG expansion in the huntingtin (HTT) gene. While most research has focused on the HTT polyGln-expansion protein, we demonstrate that four additional, novel, homopolymeric expansion proteins (polyAla, polySer, polyLeu, polyCys) accumulate in HD human brains. These sense and antisense repeat-associated non-ATG (RAN) translation proteins accumulate most abundantly in brain regions with neuronal loss, microglial activation and apoptosis, including caudate/putamen, white matter, and in juvenile-onset cases, also the cerebellum. RAN protein accumulation and aggregation are length-dependent and individual RAN proteins are toxic to neural cells independent of RNA effects. These data suggest RAN proteins contribute to HD, and that therapeutic strategies targeting both sense and antisense genes may be required for efficacy in HD patients. This is the first demonstration that RAN proteins are expressed across an expansion located in an open-reading-frame and suggests RAN translation may also contribute to other polyglutamine diseases.

Tuesday February 23rd, 2016

4:05pm - 4:40pm

SESSION II - HUNTINGTIN GENE AND GENOME: EVER CHANGING

Huntingtin functions in response to oxidative DNA damage via a novel signaling pathway that co-regulates huntingtin and P53 activity in ROS DNA-damaged cells**Ray Truant, PhD**

McMaster University

Huntingtin is a scaffolding protein involved in ER stress response, vesicle trafficking, DNA association in protein complexes, and is transcriptionally regulated by p53. The master intracellular locator and regulator of huntingtin is N17, a multifunctional domain identified as a critical sub-target within huntingtin for drug discovery in HD.

Using High Content Analysis of a natural compounds library with multi-parametric, unbiased, non-supervised machine scoring, and chemical biology studies, we have now defined an additional function of N17 as a ROS sensor. Via a single amino acid oxidation in N17, huntingtin is quickly translocated to the nucleus to sites of DNA oxidative damage. At these sites, a complex of DNA repair factors that include ATM and p53 are scaffolded to restore and monitor DNA integrity. One by-product of this DNA repair signals feedback regulation of huntingtin and p53, in a novel kinase signaling mechanism that uses an atypical triphosphate. N17 hypo-phosphorylation in mutant huntingtin is completely restored by adding back this triphosphate precursor, absent in HD due to lack of DNA repair. Restoration of this phosphorylation restores huntingtin functions, and triggers a positive cascade of events restoring DNA repair. Upon completion of DNA repair, both huntingtin and p53 protein levels are reduced. This work defines a new role of huntingtin in a DNA damage response that is affected by age-onset ROS, affects somatic CAG expansion, huntingtin protein levels, and suggests that HD is a metabolic disorder that may be corrected by dosing with the missing metabolite. These pathways outlined are consistent with HD GWAS data and reinforce or define targets for HD along one pathway of ROS damage that is consistent with the human disease, yet highlights caveats of classic mouse and cell models of HD. We present a new allelic series of human HD cell lines that have intact p53 function, and new chromobodies that allow visualization of endogenous human huntingtin in live cells during DNA repair. This natural product of oxidized DNA is a potent, non-toxic and brain penetrant therapeutic lead for HD that restores the functions of mutant huntingtin to normal.

Tuesday February 23rd, 2016

4:40pm – 5:15pm



SESSION III - TARGETED THERAPIES FOR HUNTINGTIN LOWERING**Beacons along the way – Biomarkers to monitor HTT lowering in the brain****Ignacio Munoz-Sanjuan, PhD**

CHDI

As a monogenic neurodegenerative indication, HD can theoretically be effectively treated through the suppression of mutant huntingtin expression. Toward this goal, CHDI has been actively involved in the development of molecular therapies designed to repress the HTT locus. One key limitation of all these approaches at present is the restricted brain distribution of the candidate therapeutic agents. This might limit the therapeutic efficacy of existing clinical development programs, and highlights the need for early markers that are temporally and regionally responsive to mHTT lowering. Over the last few years we have developed a set of experimental paradigms to identify proximal pharmacodynamic biomarkers to detect either mHTT itself or molecules/physiological signals that are potentially dysregulated in HD and that respond to HTT-lowering therapeutics after administration to phenotypic animals. A subset of these potential biomarkers have progressed to the level of being evaluated in human subjects. The presentation will focus on describing our strategy for the identification and development of target engagement and mechanism of action biomarkers, its current status, and future plans.

Wednesday February 24th, 2016

9:05am – 9:30am

SESSION III - TARGETED THERAPIES FOR HUNTINGTIN LOWERING

Refining experimental gene therapies for Huntington's and other diseases

Pavlina Konstantinova, PhD

uniQure

Gene therapy is one of the most advanced approaches for the treatment of neurodegenerative diseases like Huntington's disease (HD) and amyloid lateral sclerosis (ALS). For HD therapy, the mutated huntingtin (HTT) is silenced 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 a HD rat model and the humanized Hu128/21 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. For ALS gene therapy artificial miALS targeting c9orf72 were generated and evaluated for silencing efficacy. On-going work aims to evaluate the therapeutic efficacy of the miALS candidates in rodents.

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 and ALS 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. Intrathecal delivery of the vector in the minipig resulted in predominant transduction of frontal cortex, cerebellum and lumbal spine. Further studies in HD minipig model will aim to determine the level of HTT silencing in large-brained 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.

Wednesday February 24th, 2016
9:30am - 10:00am



SESSION III - TARGETED THERAPIES FOR HUNTINGTIN LOWERING**Caveats and challenges in gene silencing for Huntington's disease: Lessons from mice and monkeys****Jodi McBride, PhD**

Oregon Health and Science University

Silencing strategies that target the disease-causing gene in Huntington's disease (HD) have made remarkable headway over the past decade. A therapeutic strategy that once read more like biological fiction than reality, reduction of mutant HTT has emerged as one of the most promising therapies for this disease, with the first safety trial in human patients already underway. We, along with several other research groups from around the world, have been on a steady trajectory towards moving HTT-specific constructs from the bench to the bedside. There are myriad considerations in moving HTT-lowering strategies forward that will be discussed here, including 1) the type of silencing construct that will be employed, of which there are now several options, 2) the choice of target brain region(s) and delivery route, 3) the small and large animal models in which to evaluate efficacy of lead candidate sequences and 4) the safety of the silencing approach, which is of increased importance when partially silencing both the mutant and normal HTT allele. Here, I will discuss the lessons learned by our laboratory, in collaboration with The University of Iowa, The Children's Hospital of Philadelphia and Spark Therapeutics, as we continue to move our AAV-mediated microRNA platform forward using studies conducted in HD mouse models as well as ongoing studies in the rhesus macaque.

Wednesday February 24th, 2016

10:00am - 10:30am

SESSION III - TARGETED THERAPIES FOR HUNTINGTIN LOWERING

Development and applications of genome editing technologies

Feng Zhang, PhD

The Broad Institute of MIT and Harvard

Advances in genome sequencing technology have accelerated the rate at which we can identify genetic variants associated with phenotypes related to human health and disease, but functionally interrogating these variants remains time intensive. Being able to quickly find the causative variants in a sea of natural variation is essential to the goal of personalized medicine. To this end, new genome editing tools adapted from the microbial CRISPR-Cas system can be employed to rapidly screen through variants for functional effects as well as to model diseases based on patient-specific mutations. I discuss here how the CRISPR-Cas system can be deployed as a powerful discovery platform, highlighting recent findings from CRISPR screens, and describe therapeutic applications for this powerful tool. Finally, I present recent work exploring the next generation of genome editing technologies beyond Cas9, and how these new tools will further expand our ability to connect genotype to phenotype and, ultimately, treat human disease.

Wednesday February 24th, 2016
10:45am - 11:15am



SESSION III - TARGETED THERAPIES FOR HUNTINGTIN LOWERING**Autophagy and other pathways that protect against neurodegeneration****David Rubinsztein, PhD**

University of Cambridge

Intracellular protein aggregation is a feature of many late-onset neurodegenerative diseases, including Parkinson's disease, tauopathies, and polyglutamine expansion diseases (like Huntington's disease (HD)). Many of these mutant proteins, like that causing HD, cause disease via toxic gain-of-function mechanisms. Therefore, the factors regulating their clearance are crucial for understanding disease pathogenesis and for developing rational therapeutic strategies.

The two major intracellular protein degradation pathways are the ubiquitin-proteasome system and (macro) autophagy. Autophagy is initiated by double-membraned structures, which engulf portions of cytoplasm. The resulting autophagosomes ultimately fuse with lysosomes, where their contents are degraded.

I will briefly describe the basic biology of autophagy before outlining its roles in neurodegeneration. We showed that the autophagy inducer, rapamycin, reduced the levels of mutant huntingtin and attenuated its toxicity in cells, and in *Drosophila* and mouse HD models. We have extended the range of intracellular proteinopathy substrates that are cleared by autophagy to other related neurodegenerative disease targets, like Parkinson's disease. While autophagy induction is protective in models of various neurodegenerative diseases, certain other conditions are associated with compromised autophagy. I will discuss how two genetic variants in Parkinson's disease and Alzheimer's disease impact on autophagosome biogenesis.

Finally, I will describe two new pathways that protect against neurodegenerative disease models. One of these acts via autophagy and the other is autophagy-independent.

Wednesday February 24th, 2016
11:15am - 11:45am

SESSION III - TARGETED THERAPIES FOR HUNTINGTIN LOWERING

Selective clearance of mHtt through exploitation of the ubiquitin-proteasome pathway

Eric Reits, PhD

University of Amsterdam

The ubiquitin-proteasome system (UPS) is one of the two main degradation machineries in the cell, but its role in relation to HD is controversial. Earlier reports indicated that proteasomes were irreversibly sequestered into aggregates of polyQ-expanded mutant Htt fragments (mHtt), and that proteasomes are not able to cleave within polyQ repeats. Furthermore, it was suggested that proteasomes even become clogged by the polyQ expanded mHtt fragments.

Recently we showed that ubiquitinated mHtt is efficiently and entirely degraded by the proteasome. We also demonstrated that proteasomes remain accessible for substrates while recruited into mHtt accumulations, and are dynamically recruited into aggregates but not irreversibly sequestered. Together this indicates that proteasomes remain active and accessible for other substrates during mHtt aggregation. So why is mHtt normally not efficiently degraded?

My hypothesis is that poor recognition and/or ubiquitination of mHtt is the underlying basis for its poor breakdown. The polyQ expansion indeed affects ubiquitination of mHtt, as will be shown. This leads to the next question: which (de)ubiquitinating enzymes factors are involved in its recognition? And is there a link with the observed changes in proteasome complex formation in neuronal cells expressing mHtt?

Wednesday February 24th, 2016

11:45am - 12:15pm



FEATURED SPEAKER

Reproducibility: Crisis or opportunity?**Marcus Munafò, PhD**

University of Bristol

There have been several high-profile cases of academic fraud recently, but a more insidious threat to the integrity of science is the extent to which distortions of scientific best practice increase the likelihood that published research findings are false. There is growing evidence for a range of systemic problems within science, such as flexibility in the analysis of data, selective reporting of study results, publication bias against null results, influence of vested (e.g., financial) interests, and distorted incentive structures. These serve to reduce the likelihood that published research findings are true.

One critical problem is low statistical power. A study with low statistical power has a reduced chance of detecting a true effect, but it is less well appreciated that low power also reduces the likelihood that a statistically significant result reflects a true effect. There is growing evidence that the average statistical power of studies across a range of biomedical disciplines is very low. The consequences of this include overestimates of effect size and low reproducibility of results. There are also ethical dimensions to this problem, as unreliable research is inefficient and wasteful. Under plausible and quite conservative assumptions, it is likely that the majority of published findings are in fact false.

Improving reproducibility in neuroscience is a key priority and requires attention to well-established but often ignored methodological principles. Several opportunities exist for improving the situation, including adjusting the incentive structures within which scientists operate, improving statistical and methodological training of scientists, encouraging collaboration and independent review of study designs, and promoting open science through the pre-registration of study protocols, open data and sharing of materials. Ultimately, this will serve to maximize the scientific value of research.

Wednesday February 24th, 2016
4:30pm – 5:30pm

SESSION V - REPLACING CELLS - RESTORING NETWORKS

Phenotypic signature of HD in isogenic human embryonic stem cells

Ali Brivanlou, PhD

The Rockefeller University

We have developed a micropattern technology that allows high-resolution, quantitative and dynamic analysis of human embryonic stem cells (hESCs). When grown on micropatterns hESCs display a surprising self-organizing ability and, in response to BMP4, induce a specific signature of radially symmetrical germ layers. Using CRISPR/Cas9 genome-editing technology, we have generated a collection of isogenic Huntington's disease (HD)-hESC lines that are genetically identical to their normal counterpart except for the length of the polyQ expansion. Unexpectedly, we find that single mutations of the HTT locus change the micropattern signature in response to the same ligand. We have used this technology to screen for therapeutic compounds that revert the aberrant HD signature back to normal. The use of this phenotypic screen has led to the identification of two positive hits thus validating our approach.

Thursday February 25th, 2016

9:10am - 9:45am



SESSION V - REPLACING CELLS - RESTORING NETWORKS

A role of the protein kinase JNK3 on mutant huntingtin-induced neuropathology**Gerardo Morfini, PhD**

University of Illinois at Chicago

Cumulative evidence from numerous animal models indicate that the devastating motor and cognitive symptoms characteristic of Huntington's disease (HD) result from progressive deficits in neuronal connectivity that long preceded neuronal cell death. Accordingly, recent work from our group and others documented axonal pathology as an important pathogenic event induced by mutant huntingtin (mhtt) in vivo. Despite this knowledge, specific mechanisms and molecular components mediating axonal degeneration in HD remain unknown.

Maintenance of appropriate axonal connectivity depends upon intracellular trafficking events collectively referred to as axonal transport (AT). The unique dependence of axons on AT prompted us to evaluate whether mhtt affects this critical cellular process. Interestingly, results from these studies indicated that mhtt inhibits AT through a mechanism involving activation of the protein kinase JNK3 and phosphorylation of the major motor protein conventional kinesin. However, whether JNK3 contributes to mhtt-induced pathology in vivo remained unknown.

R6/2 mice, a widely used HD animal model, feature well-established behavioral, locomotor, and neuropathological phenotypes. Remarkably, results from studies presented at this meeting revealed a dramatic amelioration of these phenotypes upon JNK3 deletion. Moreover, a significant increase in survival was observed in R6/2-JNK3^{-/-} mice, compared to R6/2-JNK3^{+/+} mice. Providing a mechanism for these beneficial effects, quantitative fluorescence microscopic analysis studies documented a marked reduction in axonal pathology in YFP-R6/2-JNK3^{-/-} reporter mice.

Collectively, these exciting results demonstrate a major role of JNK3 on mhtt-induced neuropathology, providing a basis for the development of therapeutic strategies aimed to preserve neuronal connectivity in this disease.

Thursday February 25th, 2016
9:45am - 10:20am

SESSION V - REPLACING CELLS - RESTORING NETWORKS

Reprogramming neurons and circuits in vivo

Paola Arlotta, PhD

Harvard University

Once programmed to acquire a specific identity and function, cells rarely change in vivo. Neurons of the mammalian central nervous system (CNS) in particular are a classic example of a stable, terminally differentiated cell type. With the exception of the adult neurogenic niches, where a limited set of neuronal subtypes continue to be generated throughout life, CNS neurons are only born during embryonic and early postnatal development. Once generated, neurons become permanently postmitotic and do not change their identity for the life span of the organism. We have investigated whether neurons can be instructed to directly reprogram their identity postmitotically from one subtype into another, in vivo. We find that within a defined temporal window of “nuclear” plasticity neurons can change their class-defining traits to those of another class. Notably, afferent circuit onto the reprogrammed neurons also changes in response to direct neuronal reprogramming, adapting to the final “induced” neuronal identity. The data suggest that mammalian neurons and circuits may retain enough plasticity to change in response to appropriate stimuli and paves the way for mechanistic studies aimed at enhancing the process of neuronal conversion by extending the period of neuronal reprogramming to the mature brain. The work informs the development of new strategies to replace defined types of neurons lost to disease via the lineage reprogramming of a small number of neighboring, disease-resistant neurons of a different class.

Thursday February 25th, 2016
10:20am – 10:55am



SESSION V - REPLACING CELLS - RESTORING NETWORKS

Analyses of mood-related neural circuits in relation to Huntington's disease

Ann Graybiel, PhD

Massachusetts Institute of Technology

Affective and cognitive symptoms are early and often debilitating signs of disease progression in HD that can predominate over motor symptoms for extended periods. Our laboratory is focused on delineating, at multiple levels of analysis, mood-related circuits that likely are affected in HD patients. We are using methods in rodents and non-human primates to define circuits leading from medial prefrontal and orbitofrontal cortex to the striatum, especially to the striosomal compartment of the striatum, which is thought to be the main source of striatal projections to dopamine-containing substantia nigra neurons. Clinicopathologic evidence has suggested that striosomes may be preferentially vulnerable, relative to the surrounding matrix, in some mood-disordered HD patients.

In functional studies, our behavioral and electrophysiological work in rats and monkeys suggests that cortico-striosomal circuits could be crucial components of mood stabilizing networks engaged in a basic process underlying mood and affect: weighing the relative costs and benefits of particular actions under conditions of conflict. We are collaborating to optimize these tests for human subjects. In mice, our on-going work suggests that striosomes may have a specialized input to subsets of dopamine-containing neurons, and in monkeys, our work supports a striosome output path to the lateral habenula, a controller of dopamine and serotonin systems. These results point to striosome-based circuits as potentially critical components of networks responsible for at least some of the symptoms of anxiety, apathy, perseverativeness and related mood difficulties suffered by patients with HD and related disorders.

In Q175 work, we are generating Q175 mice with genetic tags for striosome or matrix SPNs, with the aim of developing models for therapeutic testing and assessment of compartmental function and dysfunction. To date in Q175 mice, our evidence suggests that striosomes can be progressively and regionally affected, supporting evidence in other HD rodent models. Compartmentally selective CalDAG-GEFI and CalDAG-GEFII are down-regulated in Q175 mice, as in R6/2 mice, in which we earlier found that knock-down of matrix-enriched CalDAG-GEFI promotes neuronal survival in a brain slice model of HD. Thus we hope to provide and to use cell-molecular strategies to pursue analysis of mood-related circuitry in relation to HD.

Thursday February 25th, 2016

11:10am – 11:45am

SESSION V - REPLACING CELLS - RESTORING NETWORKS

Glial as therapeutic reagents in HD

Steve Goldman, MD, PhD

University of Rochester

Glial pathology contributes to a range of neurodegenerative and neuropsychiatric diseases traditionally considered disorders of neuronal dysfunction. To explore the contribution of glial pathology to Huntington's disease (HD), we established human HD glial chimeras by neonatally engrafting the striata of immunodeficient mice with mutant huntingtin (mHTT)-expressing glial progenitor cells (GPCs), derived from either huntingtin mutant human embryonic stem cells (hESC), or from mHTT-transduced human forebrain hGPCs. Mice engrafted with mHTT hESC GPCs exhibited worse motor performance than controls chimerized with normal hESC GPCs, while striatal medium spiny neurons (MSNs) in mHTT glial chimeras were hyperexcitable and manifested high input resistance. Genomic analysis revealed that mHTT hESC GPCs exhibited the transcriptional suppression of a number of potassium channels, associated with the down-regulation of both astroglial and oligodendrocytic differentiation-linked gene expression, relative to stage-matched normal hESC GPC controls. These data suggested that glial pathology, including both astrocytic and oligodendrocytic dysfunction, might contribute significantly to HD phenotype. On that basis, we asked if the replacement of diseased glia by normal glia might slow disease progression in HD. To this end, we transplanted immunodeficient *rag2*^{-/-} x R6/2 HD (120 CAG) mice with normal, human fetal tissue-derived and CD44-isolated astrocyte-biased glia, and assessed the effects of glial engraftment on their disease progression. We found that striatal engraftment with normal glia slowed motor deterioration, rescued aspects of behavioral phenotype, and extended survival in these R6/2 mice. Electrophysiological analysis of striatal slices derived from the transplanted mice revealed that their MSNs exhibited lower input resistance and were less excitable than untreated R6/2s. Whereas R6/2 striata exhibited high interstitial K⁺, chimerization with wild-type glia normalized K⁺ levels. Together, these findings suggest a causal role for glia in HD, and indicate that the colonization of HD striata with wild-type glia can rescue aspects of neuronal function while substantially slowing disease progression, thus suggesting a new cell therapeutic strategy for disease amelioration in HD.

Thursday February 25th, 2016
11:45am - 12:20pm

SESSION VI - CLINICAL MEASUREMENTS AND MODELING**Huntington's disease measurements and models: Quality and relevance****Douglas R. Langbehn, MD, PhD**

University of Iowa

In HD, an extraordinarily diverse range of biomedical and psychological measurements have been used or proposed. One can contemplate employment of these measures in a staggering range of data models. However, the fact that one can measure some aspect of HD does not mean that one can necessarily measure it well. Further, even if measured well, it may not be very useful. Somewhat similarly, although some modeling techniques ideally may be able to unlock new insights into the complicated relationships among measurements, one often does not have the quantity or quality of data needed to exploit these potential advantages. And if appropriate data is available, a beautiful model may still only provide the right answers to the wrong questions. As with the measurements themselves, a model may or may not be useful. This may all seem rather self-evident, but experience has often reminded me how easily these considerations are under-appreciated in practice.

In the above, the meanings of “well” and “useful” depend heavily on context. For example, measurements capable of testing the presence or absence of a disease phenomenon may not have the sensitivity or stability needed to accurately quantify it. Regarding utility, precise, reliable measurements of a well-established aspect of disease are invaluable for clinical trials, though they provide no new insight into HD pathogenesis. Conversely, techniques that may shed new light on the pathophysiology of HD are often inappropriate for clinical trials.

In today's talk, I will review and distinguish the various goals that commonly drive HD studies, and I will discuss measurement and modeling criteria needed to meet these different goals. I will emphasize the (sometimes mutually exclusive) requirements for scientific discovery versus clinical trials or clinical prediction. I will also emphasize the increasing stringency requirements needed to establish the presence of an aspect of HD, quantify it, and quantify its change over a relatively short time. Throughout, I will illustrate with examples—both successes and failures—largely taken from the TRACK-HD and TRACK-ON observational HD studies.

Thursday February 25th, 2016
2:10pm – 2:50pm

SESSION VI - CLINICAL MEASUREMENTS AND MODELING

Clinical rating scales in Huntington's disease: Validation, validation, validation

Tiago A. Mestre, MD, MSc

University of Ottawa

Clinical rating scales are fundamental tools in clinical research and clinical practice. The ability to measure clinical phenomena in Huntington's disease (HD) is fundamental for the development of any treatment in HD and an accurate characterization of its natural history. Clinical rating scales need to be well-designed and appropriate for a given purpose of measurement. There are standards to assist in the development of quality clinical rating scales and determine the degree of validation and appropriateness of existing clinical rating scales. Currently, it is not known how well validated and appropriate are the clinical rating scales used in HD populations for its various purposes of measurement.

In this session, the process of development and validation of a clinical rating scale will be introduced as well as the concepts of validity, reliability and responsiveness that are core clinimetric properties assessed in a clinical rating scale. A joint effort of CHDI and the International Parkinson and Movement Disorder Society to critically assess all clinical rating scales used in HD will be presented. This project aims at characterizing how well validated and appropriate are clinical rating scales used in HD studies focusing on the core domains of motor symptoms, behavior, cognition, as well as functional ability and quality of life. The conclusions of this project will help set the priorities for clinical rating scale development and/or validation in HD, addressing the more emerging needs of a particular symptom area or purpose.

Thursday February 25th, 2016
2:50pm – 3:30pm



SESSION VI - CLINICAL MEASUREMENTS AND MODELING**Validation of a prognostic index for Huntington's disease****Jeffrey D. Long, PhD**

University of Iowa

A prognostic index (PI) for Huntington's disease (HD) is developed from variables measured at study entry to predict the occurrence of motor diagnosis, defined as the highest rating on the UHDRS Diagnostic Confidence Level. The recent availability of large HD observational databases (PREDICT, TRACK, COHORT, REGISTRY) provides an unprecedented opportunity to develop a PI and assess its generalizability in an external validation analysis. Using survival modeling for time-to-event data, a PI model was developed with the PREDICT dataset. To maximize reproducibility, different models were evaluated using leave-one-site-out cross-validation with the 32 sites of PREDICT. Results of the model development showed that a PI based on the weighted sum of the UHDRS Total Motor Score (TMS), Symbol Digit Modalities Test (SDMT), and the CAG-Age Product (CAP), had the best predictive performance among the PREDICT sites. External validation analysis consisted of using the PREDICT weights to compute the PI in three other datasets: TRACK, COHORT, and REGISTRY. External validation performance was assessed by site using Harrell's C, which indexes the extent to which the PI score predicts the timing of motor diagnosis after study entry. Using effect size standards from other fields like oncology, the results showed excellent validation performance for TRACK and COHORT, but relatively poor performance for REGISTRY. The poor performance for REGISTRY is explained by the participants being much more progressed than in the other studies. The PREDICT, TRACK, and COHORT datasets were combined (N = 1421) to determine the final PI, computed as $PI = (52 \times TMS) + (-34 \times SDMT) + (7 \times Age \times (CAG - 34))$. The results suggest the PI for HD is reproducible, having similar performance among sites in diverse geographical settings and among different research studies. The poor performance for the most progressed cohort (REGISTRY) suggests the PI predictive ability for future motor diagnosis may be limited to individuals currently in the prodromal HD stage, though the PI will still reflect advanced progression. The PI is useful for characterizing progression, forming risk groups, and selecting participants for clinical trials.

Thursday February 25th, 2016

3:50pm - 4:30pm

SESSION VI - CLINICAL MEASUREMENTS AND MODELING

Understanding Huntington's disease progression: A multi-level probabilistic modeling approach

Jianying Hu, PhD

IBM T.J. Watson Research Center

Huntington's disease (HD) is an autosomal dominant neuro-degenerative disease. The progression of HD is manifested in complex symptoms and signs both before and after clinical diagnosis, and is not well understood. Data from observational studies such as Enroll-HD, Registry and Track-HD provide an unprecedented opportunity to understand the natural history of HD. However, significant challenges remain, including how to combine heterogeneous sources of data while accounting for noises, biases, and inherent variability, and how to uncover salient patterns from heterogeneous longitudinal observations. Multi-level Probabilistic Disease Progression Modeling is a new machine learning approach for inferring disease states from longitudinal observational data. It has been applied to the modeling of chronic conditions such as COPD. In this talk I will describe our proposal to apply and expand this approach to the modeling of HD progression, with the goal of providing a comprehensive view of HD disease states, transitions, areas of manifestation and progression pathways. I will also share some early results from our efforts to combine data from Enroll-HD, Registry and Track-HD, as the first step of this comprehensive modeling exercise.

Thursday February 25th, 2016

4:30pm - 5:10pm



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POSTER ABSTRACTS

[GROUP A]

Poster Abstracts

Huntingtin:

Function/Lowering/Structure

[1]

**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Potential function for the huntingtin protein as a scaffold for selective autophagy****Joan S. Steffan**

University of California, Irvine

Although dominant gain-of-function triplet repeat expansions in the Huntingtin (HTT) gene are the underlying cause of Huntington disease (HD), understanding the normal functions of nonmutant HTT protein has remained a challenge. We report here findings that suggest that HTT plays a significant role in selective autophagy. Loss of HTT function in *Drosophila* disrupts starvation-induced autophagy in larvae and conditional knockout of HTT in the mouse CNS causes characteristic cellular hallmarks of disrupted autophagy, including an accumulation of striatal p62/SQSTM1 over time. We observe that specific domains of HTT have structural similarities to yeast Atg proteins that function in selective autophagy, and in particular that the C-terminal domain of HTT shares structural similarity to yeast Atg11, an autophagic scaffold protein. To explore possible functional similarity between HTT and Atg11, we investigated whether the C-terminal domain of HTT interacts with mammalian counterparts of yeast Atg11-interacting proteins. Strikingly, this domain of HTT coimmunoprecipitates with several key Atg11 interactors, including the Atg1/Unc-51-like autophagy activating kinase 1 kinase complex, autophagic receptor proteins, and mammalian Atg8 homologs. Mutation of a phylogenetically conserved WXXL domain in a C-terminal HTT fragment reduces coprecipitation with mammalian Atg8 homolog GABARAPL1, suggesting a direct interaction. Collectively, these data support a possible central role for HTT as an Atg11-like scaffold protein. These findings have relevance to both mechanisms of disease pathogenesis and to therapeutic intervention strategies that reduce levels of both mutant and normal HTT.

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[2]

**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Huntingtin's spherical solenoid structure enables polyglutamine tract-dependent modulation of its structure and function****Ihn Sik Seong**

Massachusetts General Hospital

The polyglutamine expansion in huntingtin protein causes Huntington's disease. Here, we investigated structural and biochemical properties of huntingtin and the effect of the polyglutamine expansion using various biophysical experiments including circular dichroism, single-particle electron microscopy and cross-linking mass spectrometry. Huntingtin is likely composed of five distinct domains and adopts a spherical α -helical solenoid where the N-terminal and C-terminal regions fold to contain a circumscribed central cavity. Interestingly we showed that the polyglutamine expansion increases α -helical properties of huntingtin and affects the intramolecular interactions among the domains. Moreover, we revealed that the polyglutamine expansion alters the phosphorylation patterns of huntingtin in a polyglutamine length-dependent manner and global dephosphorylation selectively affects the activity of mutant huntingtin. Our work provides the first glimpse into the structural properties of huntingtin and an elegant solution to the apparent conundrum of how the extreme N-terminal polyglutamine tract confers a novel property on huntingtin, causing the disease.

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[3]

**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Passive treatment with huntingtin (HTT)-specific mAb's lowers HTT levels and improves motor performance in YAC128 mice.****Oskar W. Smrzka**
AFFIRIS

HTT lowering can be achieved by indirect targeting including RNA interference, Znf-based HTT gene targeting or by targeting HTT indirectly via regulatory pathways. Direct targeting of the HTT protein per se could be achieved at the intracellular level using intrabodies [Southwell 2009] or HTT-interacting peptides [Arribat 2013]. Although HTT exists in the plasma proteome of healthy individuals [Liu 2007], the idea of extracellular mutant Huntingtin (muHTT) targeting in HD has received little attention since it is commonly regarded as a cellular release product [e.g. Weiss 2009]. However the role of extracellular (mu)HTT is not clear and it is noted that in addition to its presence in body fluids, it can also be visualized at cell membranes [Ali 2010]. A possible pathogenetic role for extracellular muHTT was suggested in pancreas [Miller 2003] and although it is established that exogenously added polyglutamine peptides have toxic impact on cell models in vitro [Yang 2002], it was only recently that muHTT was shown to propagate between cells [Cicchetti 2014; Pecho-Vrieseling 2014] and that it might have seeding properties similar to prion protein [Tan 2015]. In this context, it is not unexpected that muHTT is emerging as an extracellular CSF biomarker for monitoring HD progression and upcoming HTT lowering clinical trials [Southwell 2015, Wild 2015]. The disease-contribution of extracellular muHTT and its validation as potential antibody target remained to be defined. Therefore, we tested whether targeting extracellular Huntingtin by passive immunization with monoclonal antibodies could provide in vivo benefit. Newly developed mAb's were administered i.p. to YAC128 mice over 8 months resulting in significant reduction of muHTT in plasma and organs combined with improved motor performance. In vitro phagocytosis confirmed that these antibodies were able to mediate phagocytosis of HTT-epitope coated beads corroborating the role for clearance mechanisms. In addition to its extracellular clearance function, one of the antibodies was capable of preventing in vitro cleavage of HTT by caspase 6, making it a candidate for the development of future intrabody strategies or similar molecular targeting approaches aimed at intracellular cleavage inhibition of HTT. We conclude that muHTT is an antibody-accessible target within the extracellular milieu. It is available to antibody-based therapies including therapeutic mAb's or active vaccines (see accompanying abstract) as demonstrated by the YAC128 model. Our antibody-based approach has the potential to delay disease onset and to slow down progression of Huntington's disease.

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[4]

**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Targeting peripheral muHTT with specifically designed peptide vaccines****Oskar W. Smrzka**
AFFIRIS

Mutant Huntingtin (muHTT) spreads from one cell to another (Cicchetti 2014, Pecho-Vrieseling 2014) and shows prion-like seeding mechanisms (Yang 2002, Ren 2009, Tan 2015). HTT or muHTT can be detected in plasma of healthy and diseased individuals (Liu et al 2007, Weiss 2014), we therefore suggest that extracellular HTT lowering strategies could be addressed by antibody-based approaches, including passive and active vaccination. In an accompanying abstract we have evaluated the effects of passively administered anti-HTT antibodies in vivo thereby demonstrating for the first time that treatment of transgenic animals with particular antibodies could provide phenotypic improvement on motor functions combined with muHTT lowering in the YAC128 mouse model. In order to develop a peptide vaccine for active immunization against antibody-accessible muHTT, the N-terminus of muHTT was screened for suitable epitopes. We could identify peptides that were tested individually or in combinations of two peptides representing independent epitopes on the muHTT protein. Formulations with KLH/peptide/Alum complexes provided specific anti muHTT responses to defined targeting epitopes. Specificity, avidity, off-target binding as well as in vitro functionality were tested. Further it was tested whether new peptide vaccine formulations could provide similar beneficial phenotypic in vivo effects comparable to passively administered therapeutic HTT mAb candidates (see accompanying abstract). We found that specific peptide vaccine formulations could replicate mAb-induced therapeutic effects in YAC128 mice. Epitope analysis and functional testing of affinity purified, vaccine induced antibodies from mouse sera support our concept of peripheral muHTT targeting by antibodies and additionally suggest a significant contribution of vaccine formulation to efficacy. Antibody-accessible HTT is therefore regarded as a therapeutic target that can also be addressed by active immunization, notably by peptide vaccines. Our active vaccination strategy combines the potential to delay the onset and to slow progression of Huntington's disease with the advantage of sustained availability of antibodies in the body over long treatment periods.

Amber L. Southwell, Stefan Bartl, Christopher Kay, Michela Parth, Markus Burkert, Andreas Mayrhofer, Erika B. Villanueva, Yuanyun Xie, Louisa Dal Cengio, Seungyun Ko, Daphne Cheung, Leanna Yee, Arne von Bonin, Michael R. Hayden and Oskar W. Smrzka

[5]

**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Characterization of novel antibodies recognizing
distinct conformations of mutant huntingtin****Ali Khoshnan**

Caltech

Antibodies are instrumental for dissecting the biological functions of mutant huntingtin (HTT). Here we report the isolation and characterization of 4 new antibodies named PHP1-4. PHP1 and PHP2 interact with the proline-rich domain of HTT whereas PHP3 and PHP4 likely react with expanded polyQ. PHP1 and PHP2 display high affinity for oligomers formed by the mutant huntingtin exon-1 (HDx1) and selectively recognize protofibrils assembled in vitro. In animal models of HD, PHP1 and PHP2 detect oligomeric species of mutant huntingtin (HTT), which accumulate with age. PHP3 and PHP4 recognize monomeric mutant HTT. PHP1 and PHP2 also block the oligomerization of mHDx1 in vitro and the "prion-like" transmission of mutant HDx1 in tissue culture. The diagnostic and therapeutic properties of PHP1 and PHP2 are under investigation.

Jan Ko, Caltech Mario Isas, USC Adam Sabbough, Caltech Jung Hyun Yoo, Caltech Andreas Weiss, Evotec Douglas Macdonald, CHDI Amber Southwell, UBC Ralf Langen, USC Paul H. Patterson, Caltech Ali Khoshnan Caltech

[6]

**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Pre-clinical evaluation of allele-specific
mutant huntingtin gene silencing antisense
oligonucleotides****Amber Southwell**

University of British Columbia

Huntington disease (HD) is caused by the expansion of a CAG tract in the huntingtin (HTT) gene. The mutant HTT protein (muHTT) acquires toxic functions, and there is significant evidence that muHTT lowering would be therapeutically efficacious even if administered after the onset of symptoms. However, the wild-type HTT protein (wtHTT) serves vital functions, making allele-specific muHTT lowering strategies potentially safer than non-selective strategies. CAG tract expansion is associated with single nucleotide polymorphisms (SNPs) that can be targeted by gene silencing reagents such as antisense oligonucleotides (ASOs) to accomplish allele-specific muHTT lowering. We have evaluated ASOs targeted to HD-associated SNPs in acute in vivo studies including screening, distribution, duration of action and dosing, using a humanized mouse model of HD, Hu97/18, that is heterozygous for the targeted SNPs. We have identified four well-tolerated lead ASOs that potently and selectively silence muHTT at a broad range of doses throughout the central nervous system for 36 weeks or more after a single intracerebroventricular injection. We next conducted a preclinical therapeutic efficacy trial of lead ASOs and evaluated them for effect on the HD-like phenotypes of Hu97/18 mice. Treated mice underwent longitudinal behavioral and biochemical assessment followed by terminal neuropathology. We have demonstrated that pre-symptomatic allele-specific muHTT silencing prevents onset of behavioral HD-like phenotypes and neuropathology. Evaluation of post-symptomatic intervention is ongoing. Contingent on findings from these studies and using delivery and dosing information gained from ongoing CNS ASO clinical trials, a primary SNP-targeted ASO drug could be fairly rapidly translated for human applications.

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[7]

**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Huntingtin gene silencing in humanized mouse models of Huntington disease using allele-specific and non-selective miRNAs****Amber L. Southwell**
University of British Columbia

Huntington disease (HD) is uniquely caused by expansion of a CAG tract in exon 1 of the huntingtin (HTT) gene. HTT gene silencing, therefore, is a targeted therapeutic approach with the potential to ameliorate all aspects of HD. Previous preclinical HTT lowering studies have shown great promise in preventing onset of symptoms and even in restoring function and brain health when administered after symptom onset. The HTT protein is involved in many cellular processes throughout life, and allele-specific gene silencing approaches may be safer than non-selective approaches. Safety studies of wild-type HTT reduction in mice and non-human primates have indicated that non-selective HTT reduction will be tolerated in adults, and non-selective HTT gene silencing poses significantly fewer technical hurdles than selective gene silencing. However, the threshold of safe wild-type HTT reduction and the consequences of long-term treatment have not been sufficiently investigated. Therefore, we are developing both allele-specific and non-selective miRNA-mediated strategies for therapeutic HTT suppression. We have used AAV5 to treat Hu97/18 humanized HD model mice with allele-specific or non-selective miRNAs targeting one or both alleles of human HTT, respectively, at two months of age, the approximate time of symptom onset. Treated mice were followed for 7 months and evaluated for behavioral and biochemical changes. Following sacrifice at 9 months of age, mice were evaluated for molecular, electrophysiological, and neuropathological changes in the brain. Viral distribution and persistence were found to be excellent. HTT suppression with allele-specific miRNAs was found to be modest, resulting in modest benefit after selective suppression of mutant HTT or modest detriment after selective suppression of wild-type HTT. Treatment with non-selective miRNA was found to suppress only the wild-type HTT allele in Hu97/18 brain despite the presence of the miRNA binding site in all human HTT transcripts. Sequencing of exon 1 of the BACHD (Q97) transgene, which contains the binding site, revealed previously unreported point mutations not occurring in human HTT genes that prevent activity at this allele in Hu97/18 mice. AAV5-miRNAs were then delivered to a new humanized HD mouse model, Hu128/21, generated from YAC128 and BACWT (Q21) mice, which lack the point mutations found in BACHD mice. In Hu128/21 mice, non-selective HTT miRNAs were found to efficiently suppress both wild-type and mutant HTT. Evaluation of the therapeutic efficacy of miRNA-mediated non-selective HTT suppression in Hu128/21 mice is ongoing.

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[8]

**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Ultrasensitive measurement of mutant huntingtin in human and rodent CSF samples****Valentina Fodale**
IRBM Science Park

Huntington disease (HD) is a neurodegenerative disorder caused by a genetic mutation in Huntingtin gene (HTT) that leads to the expression of an expanded poly-glutamine protein. HD has a broad impact on a person's functional abilities including progressive motor dysfunction, cognitive decline, and psychiatric disturbances, most likely caused by both neuronal dysfunction and neuronal cell death. New therapeutic approaches to modify the disease course include a reducing the level of mutant HTT protein by gene silencing or increasing mHTT clearance. In this framework mutant HTT protein can be a biomarker for the treatment efficacy as well as disease progression, raising the necessity to develop highly sensitive immunoassays to quantify its level in biological fluids. The Single Molecule Counting (SMC™) technology, powered by Singulex®, enabled the ultrasensitive measurement of mutant HTT at levels previously undetectable in human Cerebrospinal fluid (CSF). The immunoassay was developed using the 2B7 and the MW1 antibodies that recognize the N-terminal region and the poly-Q trait of the protein, respectively, and was successfully applied to human and murine derived fluids and murine tissues. Here is presented a summary of results obtained from the assay validation process, including the evaluation of calibration curve performance, specificity, matrix effect (spike recovery, parallelism and dilution linearity), selectivity, accuracy, precision and stability.

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[9]

**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Development of a mass spectrometry-based assay for huntingtin total protein quantification and post-translational modification status determination****Sarah Elschenbroich**
Evotec

Levels and modification status of Huntingtin (HTT) protein, as the causative factor of HD, are of key interest in various disease-related contexts. For example, the total HTT level can serve as a pharmacodynamic marker to monitor efficacy of HTT-lowering strategies in a preclinical setting or in the study of animal models. The detection of post-translational modifications (PTMs) allows elucidating disease-related signaling pathways. While antibody-based detection methods exist for robust detection of HTT and some HTT PTMs, orthogonal methods based on quantitative physical measurement are highly desirable. The aim of this study is to establish a mass spectrometry-based assay, which can robustly measure levels of mutant and wild-type HTT as well as selected PTMs. The use of mass spectrometry affords accurate and sensitive quantification of multiple targets in parallel and does not depend on the existence of high quality antibodies. Specifically, multiple reaction monitoring (MRM) assays were established for total protein quantification (HTT-assay) and for phosphorylated/acylated peptides for PTM-status determination (PTM-assay). As HTT N-terminal fragments are of particular interest in the study of the protein's neurotoxicity, the MRM-HTT-assay monitors five peptides along the entire length of HTT which are conserved between human and mouse. Synthetic peptide standards were used in establishment of a customized MRM-assay, which includes optimization of both MS instrument and LC parameters. With a calibration curve experiment in mouse striatum and CSF matrix, we showed linearity of the assay over at least three orders of magnitude and limits of detection in the pM range. A protocol for tissue sample lysis and digest was optimized and spike-in experiments with recombinant Q17 and Q46 full length HTT protein showed linear performance and high recovery for both forms of HTT. Application to relevant sample groups (e.g. zQ175 vs. wild-type mice striatum) demonstrated that the established work flow allows the detection of significant changes in Htt-levels in these test samples. To further prove feasibility of our MS-based approach, we selected N-terminal PTMs of high biological interest to be included in the PTM-assay. After optimization of instrument parameters, a calibration curve experiment similar to the HTT-assay was conducted. While some peptides yielded detection limits in the pM range, assays for longer modified peptide species showed higher LODs (nM range). As levels of modified species will be low in relevant samples, we thus established a protocol for immunoprecipitation as an upfront enrichment method. Further optimization and characterization of the PTM assay is currently ongoing.

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[10]

**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Neuronal high content readouts for the DNA damage response supporting ATM as a target for HD treatment****Rhea van de Bospoort**
Charles River

The DNA damage response (DDR) is elevated in Huntington's disease (HD) suggesting contribution of the underlying molecular pathway to disease pathogenesis. The gene encoding ataxia-telangiectasia mutated (ATM) protein is a kinase involved in the DDR, apoptosis and cellular homeostasis. HD mice and patients show elevated ATM signaling and we have shown previously that ATM knockdown protected against mutant huntingtin-induced cytotoxicity in mammalian cells and transgenic Drosophila models (Lu et al, Sci Transl Med, 2014). To further develop preclinical proof-of-concept molecules targeting ATM in HD models, we used rat primary neuronal cortico-striatal co-cultures to test a tool compound set in a huntingtin-dependent cytotoxicity readout as well as a huntingtin-independent DDR readout. For the DDR readout, cortico-striatal co-cultures without huntingtin expression were exposed to the topoisomerase inhibitor etoposide at DIV6, causing DNA strand breaks by preventing re-ligation. Compounds were added simultaneously. After 1 hour, activation of the DDR was assessed by immunostaining for phosphorylated ATM (S1981). For the cytotoxicity readout, cortical and striatal neurons isolated from E18 rat embryos were co-transfected with a HTT exon 1 fragment encoding 73 glutamine (Q73) repeats and a fluorescent reporter (AcGFP for striatal neurons and AsRED for cortical neurons), before being plated on a glial feeder layer and treated with compounds. After 5 days in culture, the number of fluorescent cells were counted by an automated high content imaging and analysis platform (InCell 2200) and changes in neuronal cytotoxicity are quantified. As shown with the DDR readout, the ATM tool compounds were able to inhibit etoposide-induced p-ATM puncta formation. The cytotoxicity readout confirmed protection by various ATM inhibitors against mutant huntingtin-induced cytotoxicity, with a stronger effect in striatal neurons. In contrast, VPS34 and ATR inhibitors only showed slight protection against mutant huntingtin-induced cytotoxicity, suggesting that the protective effect of the ATM inhibitors was not modulated by VPS34 and ATR off-target effects. In conclusion, pharmacological assessment in these two assays can serve as a relevant tool to further develop ATM modulators as therapeutics for HD.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Validation of a high-throughput assay to detect
small molecule modulators of mutant huntingtin in
HD patient-derived cells****Ovadia Lazari**
Charles River

Despite the identification of the pathogenic mutation in the huntingtin (HTT) gene as the cause of Huntington's disease (HD) over 20 years ago, there remains no effective treatment. Numerous studies have been performed to identify molecules or genes which modulate the toxic effects or expression of mutant HTT (mHTT). These studies have often been based around a single hypothesis (such as reducing aggregation or preventing caspase cleavage). Historically, a sufficient and reliable assay signal, suitable for screening, had required expression of a HTT fragment, with a non-physiologically relevant polyQ repeat domain, driven off an exogenous promoter. This may have contributed to the lack of translatability of hit genes or molecules to higher disease models and reproducibility from one screen to another. Herein we present the validation of a 384-well HTS assay capable of measuring mHTT protein levels in embryonic stem cells derived from HD gene- expansion carriers. We performed a pilot screen of ~12,000 molecules from commercial sources and the CHDI library to validate the assay for hit identification, and results from this initial pilot screen will be shown. We have also developed a screening cascade of secondary and tertiary assays, including cytotoxicity assays, housekeeping protein assays, as well as assays to measure mHTT and wild type HTT in non-HD and HD-patient derived stem cells with different CAG repeats. Importantly, as the cells used in the HTS are pluripotent, we are currently attempting to create an assay in differentiated neurons from the same cellular background in order to determine whether hit compounds show similar mHTT lowering effects in a neuronal context. We aim to use these assays to identify novel compounds and biological targets responsible for mHTT modulation for the development of novel HD therapeutics.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Inhibition of the translation of huntingtin as a
therapeutic strategy to treat Huntington's disease****Serene Keilani**
Pfizer

Neurodegenerative diseases are characterized by the toxic accumulation of proteins such as mutant huntingtin (mHTT). Recent interest has shifted towards reducing the levels of these 'undruggable' pathogenic proteins. Thus, we aim to demonstrate a proof of concept that decreasing the translation of mHTT protein is a viable therapeutic strategy for the treatment of Huntington's disease. To model HD in culture, we developed a scalable and reproducible protocol to differentiate HD patient-derived induced pluripotent stem cells (HD-iPSCs) to neural progenitor cells (NPCs) and Medium Spiny Neurons (MSNs). To achieve a homogenous culture, the NPCs were sorted to enrich for PSA- NCAM (+) NPCs. The identity of the generated NPCs and neurons was confirmed with qPCR and ICC analysis. The neurons were found to express GABAergic and MSN markers. Single-cell PCR analysis confirmed the striatal identity of the produced MSNs. Furthermore, GABA release following depolarization confirmed the GABAergic identity and the capacity of the neurons to respond to stimuli. To confirm if the HD derived NPCs and neurons recapitulate the published phenotypes associated with HD, we developed assays to measure mitochondrial bioenergetics, autophagic flux, and cell death following autophagy inhibition or BDNF withdrawal. Furthermore, to gain insight into the mechanism of disease and to determine if mHTT affects translation efficiency, we performed RNAseq and ribosomal profiling on NPCs derived from an HD patient (180 repeats) and control line (H9.) The results show significant changes in gene expression and protein translation between both samples. These results are also helping us in identifying new HD-associated phenotypes. Finally, to test whether reducing the protein levels of mHTT can reverse the pathology associated with HD, we generated a stable reporter line in hESCs (H9) that contains the minimal promoter of huntingtin upstream of the reporter gene nano luciferase. We will use this reporter cell line and Pfizer's Chemogenomics library to identify mechanisms that modulate the regulation of huntingtin gene via its 5'UTR. Upon confirmation of mechanisms and pathways, the effects of the pharmacological tools can be evaluated in HD- patient derived NPCs to determine if any of the small molecules identified can rescue the cellular disease-associated phenotypes. In conclusion, we generated differentiated cells, gene editing tools, and phenotypic assays that will enable us to monitor clinically-relevant endpoints to determine if they are improved by reduced translation of mHTT.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****A role for huntingtin in the DNA damage response****Tamara Maiuri**

McMaster University

In response to many cellular stresses, the N17 domain of huntingtin is phosphorylated leading to nuclear translocation of the protein. We have previously shown that N17-phosphorylated huntingtin localizes to distinct chromatin-dependent nuclear puncta that can be altered by chemical modulation of cell signaling pathways. Given the recent findings that the DNA damage response protein ATM contributes to disease progression (Lu et al, 2014), and that GWA analysis identified DNA repair pathways as genetic modifiers of age at onset (GeM-HD Consortium, 2015), we investigated the role of nuclear N17-phosphorylated huntingtin in the DNA damage response. We show by immunofluorescence of endogenous protein and by expression of huntingtin-specific intrabodies that the protein is recruited to sites of DNA damage in a manner dependent on the kinase activity of ATM. Current experiments aim to decipher the mechanism by which huntingtin participates in the DNA damage response and the effect of polyglutamine expansion on DNA repair. We hypothesize that highly metabolically active cells such as medium spiny neurons may be susceptible to excessive oxidative damage that accumulates with age due to sub-optimal huntingtin function in the DNA repair process. Deficient repair of oxidative DNA damage could conceivably explain many HD phenotypes, including altered ATP levels due to continuous PARP activation, transcriptional changes caused by unrepaired strand breaks, somatic expansion of CAG repeats during long-patch base excision repair, and mitochondrial dysfunction due to unrepaired mitochondrial DNA.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Optimization of ELISA-based detection assays for soluble HTT on the MSD platform****David F. Fischer**

Charles River

The detection and quantitation of huntingtin protein (HTT) in select biosamples is critical to support the development of HTT lowering therapeutics for HD patients. To further optimize the existing ELISA-based assays for soluble human HTT developed on the Meso Scale Discovery (MSD®) platform, we have employed a set of monoclonal antibodies to develop even more sensitive assays. Previously, we have shown that polyQ-expanded and non-expanded HTT proteins can be quantified in a wide range of samples, including brain and muscle tissues, using a variety of ELISA-based assays. Further improvement of these assays was required in order to allow for better detection of expanded and total HTT in pre-clinical samples such as cerebrospinal fluid (CSF) and peripheral blood mononuclear cells (PBMCs). Compared to the first generation assays which employed polyclonal antibodies, the newly developed monoclonal antibody assays, CHDI_HTT_006 (detecting polyQ-expanded HTT from several species including human and mouse) and CHDI_HTT_039 (detecting human total HTT), displayed a higher signal-to-background and improved sensitivity. As a result, less tissue is required for analysis with the total protein loaded per well is 2 – 5 µg protein brain lysate, lysate from a single well containing 50k cells, or 25ul murine model CSF enabling studies that were previously not feasible. Several different sample types were tested using these novel assays: R6/2, BACHD, and Q175 knock-in mouse brain time course samples, R6/2 allelic series, mouse and human CSF, mouse brain microdialysate, mouse muscle tissue, rat primary neuron cultures, human HD patient and control PBMCs, macrophages, lymphoblasts, and fibroblasts. In conclusion, these new HTT quantification MSD assays employing monoclonal antibodies allow for improved detection of expanded and total HTT protein in a variety of different biosamples from pre-clinical and clinical studies.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Glutaminyl cyclases in Huntington´s disease:
towards genetic and pharmacological
proof-of-concept in BACHD mice****Stephan von Hörsten**

Friedrich-Alexander-University

Background: There is evidence and several hypotheses for a role of glutaminyl cyclase (QC, QPCT) and its isoenzyme (isoQC, QPCTL) in the pathology of Huntington´s disease (HD). Firstly, posttranslational modifications of Huntingtin (HTT) have been shown to affect its biophysical and neurophysiological properties. Among those modifications, pyroglutamate HTT (pEx-HTT) may be formed via cyclization of truncated HTT species by enzymatic QC/isoQC activity. This mechanism could enhance pathologic processes, similar to what has been proven in models of beta- amyloid (A β) toxicity, also affecting the striatum (Becker et al., 2013). Secondly, subclinical neuroinflammation in HD may be triggered and sustained via CCL2/MCP- 1, a chemokine, which matures via isoQC-dependent N-terminal pyroglutamate formation (Cynis et al., 2011). Thirdly, the QC mediated induction of increased alphaB-crystallin (Cryab) chaperone levels may inhibit HTT toxicity (Jimenez- Sanchez et al., 2015). We therefore initiated genetic and pharmacological proof-of- concept (POC) experiments targeting QC/isoQC in transgenic mouse and rat models of HD. Methods: Pilot experiments aiming at genetic POC were initiated by crossbreeding QC ko (Schilling et al., 2011) or isoQC ko (Becker et al., 2016) mice with BACHD mice (Gray et al., 2008). Pharmacological POC is aimed by (a) tolerability and dose- response studies in C57BL/6N using a QC/isoQC inhibitor PQ912 (Probiobdrug AG[®], presently in clinical phase 2 in Alzheimer´s disease patients), followed by (b) early and (c) late interventional studies in BACHD mice and tgHD rats (von Hörsten et al., 2003). Motorfunction (RotaRod, Catwalk), startle PPI, emotionality (LDT, SI) as well as automated- intra-home-cage activity and metabolic performance (Urbach et al., 2014) are behavioral end points to be examined. Cell numbers, striatal volume, DARPP32, Cryab, CCL2 are considered as the neuropathological endpoints. Results: So far, pilot data illustrate that crossbreeding of isoQC ko mice ameliorates the motor functional and intra-home-cage phenotype in BACHD mice and that PQ912 is well tolerated in wide range of dosages with no obvious adverse effects. Studies are ongoing. Update will be provided. Conclusions: Glutaminyl cyclases (QC, QPCT) probably represent a drugable target also in HD.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Transglutaminase 6 in Huntington´s disease: role in
mutant huntingtin aggregate formation****Stephan von Hörsten**

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Objectives: Transglutaminases (TGs) are multifunctional proteins with distinct enzymatic activities. In mammals, at least eight isoforms are known, of which only four (TG1, TG2, TG3 and TG6) are found in the brain and TG6 representing the neuronal isoform. TGs share the common feature of catalyzing irreversible modifications on proteins, including the acyl-transfer between glutamines and lysines. Under physiological conditions, this transamidation reaction is latent because of low free Ca²⁺ levels. In Huntington´s disease intracellular free Ca²⁺ may rise thus favoring the crosslinking activity of TGs to the expenses of their GTP-binding function, which are mutually exclusive. Accordingly, there is evidence for a role of mammalian TGs in Huntingtin (HTT) processing, but the role of TG6 in Huntington´s disease (HD) is less well characterized. Material and methods: The present study was outlined to investigate expression, distribution and activity of transglutaminases (Schulze-Krebs et al., 2015) in Huntington´s disease in BACHD mice (Gray et al., 2008) and tgHD rats (von Hörsten et al., 2003). We were particularly interested in analyzing the involvement of TG6 in the age- and genotype-specific pathological progressions by protein, histological and functional assays. Experiments aiming at a genetic proof-of-concept by crossbreeding TG6 ko mice (Tgm6tm1a(KOMP)Wtsi; Sanger Institute) with BACHD were initiated. Results: We demonstrate the physical interaction between TG6 and mutant huntingtin (mHTT) by cross-linker analysis. Using a newly development TGs in situ activity assay (Schulze-Krebs et al., 2015), we found very clearly a TG6 specific signal in the cortex of BACHD mice after incubation with a TG6-specific biotinylated peptide in the presence of free Ca²⁺. Similar results were also obtained in >12 month old tgHD rats. In this model, TG6 expression and activity were especially abundant in the olfactory tubercle and piriform cortex, the regions displaying the highest amount of mHTT aggregates in tgHD rats. Furthermore, mHTT aggregates were colocalized within TG6-positive cells. Experiments using BACHD x TG6 ko mice are presently ongoing. Conclusion: These findings yet point towards another important post-translational process involved in the neurobiology of HD with a prominent role for TG6, potentially promoting mHTT aggregate formation.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****An intein-based strategy for the production of tag-free huntingtin Exon 1: new opportunities for understanding the structural and molecular determinants of huntingtin aggregation****Sophie Vieweg**

Ecole Polytechnique Fédérale de Lausanne

The increased levels and aggregation of N-terminal Huntingtin protein (Htt) fragments have been implicated in Huntingtin toxicity and the pathogenesis of Huntington's disease (HD). The first exon of the Huntingtin protein (Httex1) is one of the most actively studied Htt fragments because its overexpression in R6/2 transgenic mice has been shown to recapitulate several key features of HD. However, the majority of biophysical studies of Httex1 are based on assessing the structure and aggregation of fusion constructs where Httex1 is fused to large proteins, such as glutathione- S-transferase (GST), maltose-binding protein (MBP) or thioredoxin (TRX), upon in situ cleavage of these proteins. Herein, we report an intein-based strategy that allows, for the first time, the rapid and efficient production of native tag-free Httex1 with polyQ repeats ranging from 7-49Q. Biophysical characterization of these proteins revealed that they show the expected structural and aggregation properties of tag-free Httex1 proteins produced by chemical or semisynthetic strategies, but exhibit a distinct aggregation behavior compared to some Httex1 fusion proteins or Httex1 proteins produced by in situ cleavage of fusion proteins. The ease of production and availability of tag-free Httex1 in milligram quantities should advance future structural and functional studies, and facilitate the development of assays for the identification of Httex1 binding ligands or proteins to modulate Htt aggregation and toxicity.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Detection of the aberrantly spliced exon 1 – intron 1 HTT mRNA in HD patient post mortem brain tissue and fibroblast lines.****Gillian P Bates**

King's College London

We have previously shown that exon 1 of the huntingtin gene does not always splice to exon 2 resulting in the production of a small polyadenylated mRNA that encodes an exon 1 HTT protein (1). This read-through product is generated in all knock-in mouse models of HD carrying CAG repeats in the mutant range (≥ 50), utilizing cryptic polyadenylation sites located at 677 and 1145 into mouse intron 1. The level of aberrant splicing is proportional to the length of the CAG repeat. The read-through product is also readily detected in the YAC128 and BACHD mouse models with a cryptic polyadenylation site that is located 7327 bp into human intron being used in both models. Over the past 20 years, multiple model systems have shown that mutant versions of exon 1 HTT are highly pathogenic and this N-terminal HTT fragment may be a major factor in the initiation and progression of HD pathogenesis. However, the presence of this product in fragment in HD patient tissues was not easy to detect using the assays that we initially developed. We have now established a set of qPCR assays that quantify sequences located close to the cryptic polyadenylation site in human HTT intron 1. These readily detect the exon 1 – intron 1 HTT mRNA in the somatosensory cortex, hippocampus and cerebellum of post mortem brains from individuals with juvenile HD. These human HTT intronic sequences are also present in fibroblast from juvenile HD patients carrying highly expanded CAG repeats. We shall also present data on changes in the relative abundance of the exon 1 – intron 1 Htt mRNA in a knock-in mouse model with disease progression. The technical challenges in detecting human HTT intron 1 sequences will be discussed. (1) Sathasivam K*, Neueder A*, Gipson TA, Landles C, Benjamin AC, Bondulich MK, Smith DL, Faull RLM, Roos RAC, Howland D, Detloff PJ, Housman DE, Bates GP (2013). Aberrant splicing of HTT generates the pathogenic exon 1 protein in Huntington's disease. *Proc. Natl. Acad. Sci.* 110, 2366-2370.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****High content analysis identifies kinetin in restoration of a function of mutant huntingtin protein in Huntington's disease****Laura Bowie**
McMaster University

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by a polyglutamine tract expansion in the huntingtin protein, which interferes with its normal biological functions in stress response and causes cellular toxicity. An important modulator of this toxicity is the phosphorylation state of two critical serine residues in the huntingtin protein. Mutant polyglutamine-expanded huntingtin is hypophosphorylated, and restoration of this phosphorylation can prevent, or dramatically reverse, disease in mouse models of HD. We conducted an HCA screen on a small natural compounds library. Wells were imaged automatically and scored using a non-supervised, machine-based algorithm (Phenoripper). From this screen, we identified compounds that were effective at modulating huntingtin phosphorylation state and/or localization. One of these effective natural products was kinetin (N6-furfuryladenine). Kinetin is metabolized to an ATP analog (KTP) that can act as a phospho-donor for certain kinases. Casein kinase 2 (CK2) can utilize KTP to phosphorylate huntingtin N17. Low doses of kinetin restore phosphorylation of mutant huntingtin in HD model cells, and are cytoprotective in these cells under stress conditions. Kinetin is produced at sites of DNA damage, where it co-localizes with the necessary cellular proteins to convert it to KTP, as well as CK2 and p53 S392p. We hypothesize that kinetin is acting as a phospho-donor for CK2 at sites of DNA damage in a novel, endogenous oxidative DNA damage response pathway. This pathway is disrupted in HD cells due to improper excision of kinetin, and is restored by addition of exogenous kinetin. The efficacy of this compound on huntingtin function is promising and presents a proof-of-principle that non-supervised machine sorting can detect subtle phenotypes, leading to new chemical biological tools and a potential novel therapeutic for HD.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Toward developing a personalized allele-specific gene silencing therapy for Huntington disease****Nicholas S. Caron**
University of British Columbia

In HD, mutant huntingtin is unable to perform many of its normal functions and acquires additional toxic functions that disrupt multiple vital cellular pathways. Both the loss and gain-of-functions associated with the polyglutamine expansion causes cellular dysfunction that ultimately leads to the neurodegeneration observed in HD. Therefore, reducing levels of mutant huntingtin in patients represents an attractive therapy for HD. Huntingtin lowering can be achieved through non-selective silencing of total HTT or by selectively silencing the mutant allele. To date, gene silencing strategies have undergone extensive preclinical evaluation as therapies for HD. Non-selective silencing using antisense oligonucleotides (ASOs) or RNA interference methods have been shown to improve behavioural and neuropathological phenotypes in multiple animal models of HD. These studies suggest that reduction of normal huntingtin is safe and well tolerated. However, huntingtin has numerous cellular functions and is essential for neuronal health, and it remains unclear whether its sustained reduction will be tolerated over the long treatment durations likely required in humans. Therefore, selectively lowering mutant huntingtin in patients may represent a safer, more direct therapeutic approach for HD. One strategy to selectively inhibit the production of mutant huntingtin is to silence mutant HTT using ASOs targeted to single nucleotide polymorphisms (SNPs) at the HTT locus that are strongly associated with the CAG expansion (HD-SNPs). Our lab has previously evaluated ASOs targeted to one such HD-SNP and identified potent, selective, and well tolerated candidates currently undergoing pre-clinical validation. However, targeting any individual HD-SNP would only provide a treatment option for a portion of the HD population. Therefore, we are developing a panel of ASOs targeting HD-SNPs specific to the three most common HD HTT haplotypes to maximize the number of HD patients that could be treated through selective silencing of mutant HTT. Using this approach, each individual ASO drug in the panel would treat a distinct subset of the HD population and in combination would provide a personalized treatment option for the majority of HD patients. We have identified and validated eight of the most common HD-SNPs in HTT and we are currently screening ASOs in patient-derived cell lines to identify potent and selective molecules to advance to efficacy studies in neurons and glia differentiated from HD patient induced pluripotent stem cells (iPSCs). If effective in iPSC differentiated cells and tolerated in animal models of HD, this personalized therapy could be rapidly translated into a similar approach for humans.

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[21]

**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Semisynthesis of mutant (43Q) Httex1 enables investigation of the crosstalk between N-terminal phosphorylation and acetylation in regulating the aggregation of exon1 of the huntingtin protein****Anass Chiki**

Ecole Polytechnique Fédérale de Lausanne

Huntington's disease (HD) is a fatal genetic neurodegenerative disorder caused by a CAG repeat expansion (more than 36 repeats) of the huntingtin gene which, is translated into a polyglutamine (polyQ) stretch within the first exon of the Huntingtin protein (Httex1). In HD patient brains, the expanded Huntingtin protein (Htt) abnormally aggregates in the striatum and cortex causing neuronal dysfunction and death. The Htt protein undergoes a range of post-translational modifications (PTMs), which have been shown to modulate the toxicity and aggregation properties of the protein. Therefore, a better understanding of the effects of these PTMs on the biophysical and biochemical properties of Httex1 is essential for understand the function of Htt in health and disease. In this study, we developed a novel semisynthetic methodology for the site-specific introduction of single and multiple PTMs into Httex1. Using optimized chemical ligation and desulfurization conditions, we produced for the first time, phosphorylated (pT3) and acetylated (at K6, K9 and K15) mutant Httex1 in mg quantities and compared their aggregation properties to unmodified mutant Httex1. We found that T3 phosphorylation (pT3) dramatically inhibits the aggregation of mutant Httex1, an affect that was not reproduced by the phosphomimetic mutation (T3D), demonstrating that this mutation poorly reproduces the effect of phosphorylation in vitro. Our results also show that acetylation at K6, K9 or K15 had no effect on the aggregation properties of Httex1. Interestingly, K6 acetylation reverses the inhibitory effect of T3 phosphorylation, suggesting possible crosstalk between the two PTMs. The possibility to introduce authentic post-translational modification into mutant Httex1 by semisynthesis in the absence of large protein fusions will enable us to accurately determine the role of different PTMs in modulating the aggregation, structure and function of Htt in health and disease.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Dissecting the role of N-terminal serine phosphorylation (S13, S16 and S13/S16) in regulating the aggregation of the huntingtin exon1 protein****Sean DeGuire**

Ecole Polytechnique Fédérale de Lausanne

Huntington's disease (HD) is a progressive neurodegenerative disease caused by a polyglutamine (polyQ) repeat expansion mutation and is characterized by aggregation of huntingtin (htt) in intranuclear inclusions. Observed phosphorylations at T3, S13, and S16 of htt demonstrate diverse effects on HD pathology. Phosphorylation at these residues may play a central role in modulating htt structure, function, and toxicity by altering htt structure but ideal tools to study those effects are currently unavailable. We have addressed this limitation by preparing a series of semisynthetic proteins with site-selectively introduced phospho-serine residues or phosphomimetic mutations (S↔D) at S13 and/or S16 of wild type (22Q) and mutant (42Q) huntingtin exon1 (Httex1) and examined their biophysical properties. Analysis of the in vitro aggregation kinetics of these proteins revealed a rate inhibitory role on both wild type and mutant Httex1 aggregation for serine phosphorylation at either position. Phosphorylation at both residues resulted in greatly decreased aggregation rates and significantly altered aggregate morphology. By contrast, phosphomimetic mutation at the same sites imparted a weakly inhibitory effect on Httex1 aggregation that was unobservable at disease relevant polyQ repeat lengths. Studies that rely heavily upon such mutant proteins to assess the effects of htt phosphorylation in vivo may fail to reproduce the structural consequences of htt phosphorylation. These results highlight the need to identify targetable proteins or cellular pathways responsible for the regulation of htt phosphorylation in vivo. Furthermore, the semisynthetic proteins prepared herein are ideal tools for use in studies aimed at this identification.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Probing structure and conformation of huntingtin exon1 using single molecule FRET****John B. Warner**

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Huntington's disease (HD) results from an expansion of the poly-glutamine (polyQ) tract in the Huntingtin protein (Htt) resulting in protein misfolding, aggregation, and cellular toxicity. The N-terminal Exon1 fragment (Httex1) has been identified as a minimal subunit required to recapitulate key features of the human pathology and was shown to regulate Htt toxicity and cellular properties in different cellular and animal models of HD. However, very little is known about the structural properties of this protein and the structural basis underlying its role in HD pathogenesis owing to the highly dynamic nature of the protein and its high propensity to aggregate when the polyQ repeat exceeds the pathogenic threshold of 36Q, thus making it difficult to probe the structural conformation of Httex1 using classical structural biology techniques (e.g. protein crystallography and cryo-electron microscopy). Single molecule fluorescence resonance energy transfer (smFRET) can provide structural and conformational information of proteins in solution at physiologically relevant concentrations. Using recently developed chemical and semisynthetic strategies developed in our laboratory, we produced a library of 15 site-specific dual fluorophore labeled Httex1 to probe the structural effect of the polyQ domain expansion and post-translational modification on the conformation of Httex1 at the single molecule level using smFRET. Though unstructured and unfolded, Httex1 is well solvated contrary to previously published work using polyQ model peptides. Our data shows that as the polyQ repeat length increases there is an increase in the compaction of Httex1 until the pathogenic threshold is reached. The change in Httex1 conformation observed using smFRET is consistent with the hypothesis of a 'molecular switch'. Detailed structural and conformational data on Httex1 will provide crucial insight into the relationship between protein aggregation and HD pathogenesis. In addition, our smFRET assay provides unique opportunities to evaluate the binding of small molecules, peptides, or proteins to Httex1 under conditions that mimic its physiological state in an effort to discover novel strategies for stabilizing the protein and preventing its aggregation and toxicity.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Peripheral HTT silencing as a therapeutic strategy in HD****Jeffrey B. Carroll**

Western Washington University

Although Huntington's disease (HD) is primarily conceived as a neurodegenerative disorder the causative agent of HD, mutant huntingtin protein (mHTT), is ubiquitously expressed. Consistent with this wide expression profile, a range of peripheral phenotypes are observed in human HD mutation carriers and animal models, though the relevance of these phenotypes is not yet clear. Brain-body interactions are increasingly appreciated in neurological diseases - Alzheimer's disease progression, for example, is exacerbated by peripheral immune activation. Hepatic encephalopathy, the neurological sequelae of liver failure, results in alterations in corticostriatal circuitry reminiscent of HD patients. In theory, it is possible that peripheral expression of mHTT causes sufficient dysfunction to appreciably impact the course of the disease in the CNS and that modulating disease progression by targeting peripheral cells is possible. To test this specific hypothesis, we peripherally administered antisense oligonucleotides (ASOs) that target mutant and wild type murine Htt for degradation. Female HttQ111/+ mice were treated from 2 months of age with intraperitoneal injections of 50 mg/kg/week of pan-Htt ASO (ASO1), non-targeting control ASO (control), or saline. By Western blot, we achieved >90% knockdown of HTT in the liver between 2-10 months of age. Mice were sacrificed at 10 months of age, and we are currently characterizing a number of molecular endpoints in both peripheral and central tissues to determine whether this intervention is associated with any amelioration of HD-related phenotypes. To enable this effort, we developed a battery of endpoints that are sufficiently powered to evaluate the efficacy of peripheral HTT silencing in B6.HttQ111/+ mice. We will present histological quantification of p62- positive mHTT aggregates and DARPP-32 levels in the dorsolateral striatum, as well as preliminary transcriptional profiling of the striatum using QRT-PCR, for a range of genes altered by expression of mutant Htt.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Characterization and functional validation of post-translational modifications (PTMs) of endogenous huntingtin (Htt) protein****Tamara Ratovitski**

Johns Hopkins University

PTMs of expanded Htt are important modulators of HD pathogenesis. Previously, we have discovered many novel sites of PTMs on the endogenous mouse and human Htt, and have implicated several sites in pathogenesis. We find that the PTMs are present in clusters, which are mostly located in the unstructured regions between the predicted HEAT repeats. We developed functional assays for studying the relevance of these PTMs to HD, using mass spectrometry for quantifying PTM stoichiometry, and cell biological read-outs for determining cellular effects. To evaluate the stoichiometry of PTMs, we established a new method, based on stable isotope labeling using isobaric tandem mass tag (TMT). Synthetic modified peptides are spiked with the samples to increase the probability of detecting low abundant endogenous peptides, containing the PTMs of interest, which may be missed during MS analysis due to poor ionization or fragmentation or data dependent sampling. As a result, we were able to detect significant differences in several targeted PTMs between HD and WT mouse tissues. We generated a series of expression constructs with PTM alterations within full-length Htt, which provides the opportunity to compare cell biology of the pathogenic versus non-pathogenic constructs to better define important cellular pathways (versus epiphenomenal effects) in HD pathogenesis. As our primary screen, we are using neuronal death measured by nuclear condensation. We found that some of the PTM alterations (S421A, K1190R, S1201A, K2548R) showed reduced toxicity in cortical neurons, whereas other alterations showed a toxicity similar to unmodified expanded Htt. We also developed a number of new assays to assess the effects of PTMs on Htt co-localization with cellular organelles. We are using fluorescent dyes to assess the effects of PTM alterations on mitochondrial depolarization and mitochondrial swelling, as functional read-outs of mitochondrial function. We are expanding our studies of PTMs of Htt towards other modifications, particularly methylation and dimethylation. We discovered around 30 novel arginine methyl/dimethyl sites on full-length expanded Htt purified from transfected HEK293 cells. We found that there are clusters of PTMs (phosphorylation, acetylation, and methylation) positioned near each other within several regions of Htt, providing a potential for PTM crosstalk, which may regulate Htt function, and modulate cellular toxicity of expanded Htt. Further studies will be directed towards better understanding of "PTM code" within Htt, and the role of PTM crosstalk in HD. Identifying the enzymes catalyzing the most relevant PTMs will provide attractive therapeutic targets for HD.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****SUPT4H selectively regulates the expression of genes containing expanded CAG repeats and is a potential therapeutic target against neurodegenerative Huntington's disease****Tzu-Hao Cheng**

National Yang-Ming University

Production of protein containing lengthy stretches of polyglutamine encoded by multiple repeats of the trinucleotide CAG is a hallmark of Huntington's disease (HD) and of a variety of inherited neurological disorders. Our earlier work has shown that interference with production of the transcription elongation protein SUPT4H results in decreased cellular capacity to transcribe mutant huntingtin gene (Htt) alleles containing long CAG expansions, but has little effect on expression of genes containing short CAG stretches. zQ175 and R6/2 (two animal models for HD) are genetically engineered mouse strains whose genomes contain human HTT alleles that include greatly expanded CAG repeats. Here we show that reduction of SUPT4H expression in brains of zQ175 mice by intracerebroventricular bolus injection of antisense oligonucleotides (ASOs) directed against Supt4h, or in R6/2 mice by genetic deletion of one copy of the Supt4h gene, results in a decrease in mRNA and protein encoded specifically by mutant Htt alleles. We further show that reduction of SUPT4H in mouse brains is associated with decreased HTT protein aggregation, and in R6/2 mice, also with prolonged lifespan and delay of the motor impairment that normally develops in these animals. Our findings support the view that targeting of SUPT4H function may be useful as a therapeutic countermeasure against HD.

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**GROUP A: HUNTINGTIN:
FUNCTION/LOWERING/STRUCTURE****Optimized scalable semi-synthesis by native
chemical ligation of post-translationally modified
huntingtin exon1 [2-90] fragments with one or
more phosphorylated residues in the Nt17 sequence****Finotto Marco**

IRBM Science Park

Huntington disease (HD) is a neurodegenerative disorder caused by an expansion of polyglutamine repeats within the exon1 region (HTT ex1) of the N-terminal domain of the huntingtin protein (HTT), resulting in loss of normal wild-type function, gain of toxic function, and enhanced HTT aggregation. The exon 1 region of HTT contains an N-terminal sequence of 17 amino acids, Nt17, which plays an important role in the function of the protein and in protein aggregation. The Nt17 region can undergo posttranslational modifications (PTMs), including phosphorylation and acetylation, that influence the behavior of the protein. Studies on how PTMs affect the conformation and consequently the function of HTT in HD offer great potential in terms of identifying a path forward to therapeutic intervention. Therefore, elucidating the role of the N-terminal region of huntingtin on its oligomerization, aggregation, and toxicity is absolutely required and particular emphasis has been put on the study of post translational modifications such as phosphorylation at specific residues (T3, S13 and S16) of the Nt17 region. The aim of this work was to synthesize a series of HTT exon1 mutants, spanning region 2-90, with a 22Q polyQ domain consistent with the normal non- pathological sequence, and single or multiple phosphorylated residues at T3, S13 and S16. This effort was accomplished by means of a process initially developed in the Lashuel group: 1) chemical synthesis of N-terminal Nt17 sequences containing one or more phosphorylated residues; 2) recombinant expression of an HTT exon 1 protein precursor, AA 18-90, with a Q18C mutation incorporated by intein splicing; 3) native chemical ligation (NCL) of the different Nt17 peptide fragments with the protein precursor followed by desulfuration of C18 to A18. Key factors for the success of this project were: a) the optimization of the chemical process for the production of the PTM peptides HTT[2-17] obtained as C-terminal N-acyl-benzimidazolinone (Nbz) precursors for the NCL; b) the optimization of the ligation reaction steps during the protein synthesis process. In this study we demonstrate the scalability of this process by the synthesis of a series of PTM HTT exon1[2-90] proteins in amounts (>10 mg) sufficient to support both crystallization studies and bioassay development.

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[GROUP A]

Poster Abstracts

HD Biological Mechanisms

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GROUP A: HD BIOLOGICAL MECHANISMS

Exosomal huntingtin in the peripheral blood of R6/2 and zQ175 HD transgenic mice**Manho Kim**

Seoul National University Hospital

Exosomes are the smallest vesicles (40-100 nm) and secreted by diverse cell types. Nanovesicles are found in various body fluids. Depending on their parental origin, exosomes contain a variety of molecules- collectively termed as "cargo" contents such as proteins, lipids, non-coding RNAs, mRNA, and miRNA, and are delivered to the surrounding or the distal cells. Small amounts of mHtt can be detected by ultrasensitive single-molecule counting (SMC) mHtt immunoassay. We investigated exosomal mHtt derived from conditioned media of in vitro HD model by the MAB1574 antibody, but not by MAB5374. Exosomal mHtt from serum of R6/2 and zQ175 HD mouse is also detected by the MAB1574. Our results provide a clue to detect mHtt in exosomes. And further studies to investigate exosomal mHtt derived from CSF will be needed.

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GROUP A: HD BIOLOGICAL MECHANISMS

Dysregulated gasotransmitter and cGMP signaling in Huntington's disease**Bindu D. Paul**

Johns Hopkins University

The second messenger cGMP participates in diverse physiological processes ranging from vasorelaxation in the peripheral tissues to modulation of cognitive functions and synaptic plasticity in the nervous system. The concentration of this cyclic nucleotide is maintained by a fine balance between the activity of its biosynthetic enzyme, guanylyl cyclase, and the cGMP phosphodiesterases which degrade it. Gaseous signaling molecules or gasotransmitters such as nitric oxide (NO), hydrogen sulfide (H₂S) and carbon monoxide (CO) have been shown to regulate cGMP disposition. While NO and CO activate guanylyl cyclases to stimulate cGMP production, H₂S inhibits phosphodiesterases as well as activates guanylyl cyclases to elevate cGMP levels. Dysregulated cGMP signaling has been observed in Huntington's disease (HD), which is characterized by expansion of polyglutamine repeats in the protein huntingtin, affecting multiple cellular processes leading to motor and cognitive deficits. Treatment of HD mouse models with cGMP specific phosphodiesterase (PDE) inhibitors mitigates these abnormalities. We sought to understand the molecular basis of cGMP signaling in healthy and neurodegenerative conditions. We measured cGMP levels in mice lacking cystathionine gamma-lyase (CSE), neuronal nitric oxide synthase (nNOS) and heme oxygenase 2 (HO-2), the biosynthetic enzymes for H₂S, NO and CO respectively. Altered H₂S biosynthesis has been observed in HD, which contributes to neurodegeneration. While the nNOS and HO-2 knockout mice exhibited modest decreases in cGMP levels, the CSE knockout mice displayed significant reductions in the cyclic nucleotide. We monitored the expression and activity of the biosynthetic enzymes of these gases in HD models. The expression of CSE, nNOS and HO-2 were downregulated in HD with a concomitant decrease in the production of these gases in the Q175 mouse model of HD. In addition, patterns of sulfhydration and nitrosylation, posttranslational modifications mediated by H₂S and NO, pertaining to cyclic nucleotide signaling pathways were altered in HD. Taken together our results reveal an interplay between these three gasotransmitters to regulate cGMP signaling that involves both cooperative as well as compensatory mechanisms. References 1. Paul BD, Sbodio JI, Xu R, Vandiver MS, Cha JY, Snowman AM, Snyder SH. *Nature* 2014, 509:96-100. 2. Paul BD* and Snyder SH*. H₂S: a novel gasotransmitter that signals by sulfhydration. *Trends in Biochemical Sciences* 2015, 40:687-700 (*Co-corresponding author). 3. Paul BD and Snyder SH. *Protein Sulfhydration. Methods in Enzymology* 2015, 555:79-90. 4. Paul BD and Snyder SH. *Antioxidants & Redox Signaling*. 2015, 22:411-423.

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GROUP A: HD BIOLOGICAL MECHANISMS

Altered endocannabinoid-mediated excitatory synaptic plasticity in the striatum in a Huntington's disease mouse model**Lynn A. Raymond**

University of British Columbia

Animal models of Huntington's disease (HD) show altered cortical-striatal presynaptic glutamate release and trafficking/signaling of postsynaptic glutamate receptors, changes which often precede the motor phenotype. Synaptic proteins form interaction hubs with wild-type huntingtin (Htt), and we hypothesize that early alteration of these interactions impact plasticity of excitatory synapses onto striatal medium-sized spiny projection neurons (SPN) in HD mice. We recorded from striatal SPN in acute brain slice, stimulating excitatory afferents to test the response to synaptic plasticity-inducing protocols, comparing YAC128 with wild-type (WT) FVB/N mice crossed with mice expressing eGFP in D2-dopamine receptor-expressing SPN. Long-term depression (LTD) of excitatory postsynaptic current or field potentials, induced by 100Hz stimulation (high frequency stimulation - HFS), was robust in striatal SPN from WT mice but significantly attenuated in YAC128 mice; the difference was most pronounced at cortical afferents onto D2-SPN, which are more vulnerable in early HD. HFS-LTD in WT SPN was associated with an increased paired-pulse ratio (PPR), consistent with a presynaptic locus for synaptic depression, whereas no change in PPR was observed after HFS in YAC128 SPN. Previous studies indicate that HFS-induced striatal LTD is mediated by retrograde endocannabinoid signaling from SPN to presynaptic cortical cannabinoid receptor-1 (CB1). Consistent with this, our pharmacological experiments in slice and immunocytochemical data from cortical-striatal co-cultures suggest reduced numbers/function of CB1 in YAC128 cortical neurons. On the other hand, short-term plasticity – Depolarization-induced Suppression of Excitation (DSE) – also mediated by CB1, was intact at cortical synapses onto SPN in YAC128, suggesting that presynaptic CB1 receptor alterations differentially impact signaling in long- vs. short-term plasticity at cortical-striatal synapses. Strikingly, HFS-LTD was restored by augmenting levels of endocannabinoid 2-arachidonoyl-glycerol. These results will increase understanding of early cognitive impairment, as well as striatal vulnerability to excitotoxicity, in HD. Thanks to Dr. Ken Mackie, Indiana University, for the CB1 antibodies. Supported by CHDI Foundation and Canadian Institutes for Health Research.

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GROUP A: HD BIOLOGICAL MECHANISMS

A pathogenic interaction between complement and microglia drives early loss of synapses in Huntington's disease**Daniel Wilton**

Boston Children's Hospital

Synapse loss is a hallmark of many neurodegenerative diseases including Huntington's disease HD (Graveland et al., 1995; Ferrante et al., 1991; Mucke et al., 2012). HD pathology involves reduced synaptic transmission in the basal ganglion and loss of synaptic proteins. Despite synaptic dysfunction being one of the earliest events in HD pathology, the mechanisms driving synapse loss remain unknown. During development, synapse loss as a result of pruning is a normal and highly regulated process required for the correct wiring of the brain. Our laboratory along with others have shown that microglia play a key role in regulating this process (Schafer et al., 2012; Paolicelli et al., 2011). In the developing mouse visual system, microglia phagocytose synapses that are undergoing pruning in a manner that is dependent on complement (C1q, C3 and CR3) and neuronal activity (Stevens et al., 2007; Schafer et al 2012;). Our data suggest a model in which less active synapses are selectively labeled with complement and then engulfed by microglia that express complement receptors (CR3/Cd11b). In Huntington's disease, microglial activation is an early event in HD pathology and upregulation of complement components has been detected in post-mortem tissue. We hypothesize that the developmental mechanism of microglia-mediated synaptic pruning is aberrantly reactivated early in Huntington's disease (HD) pathology and mediates early synapse loss and dysfunction. In support of this hypothesis, preliminary data from two HD models (BACHD, zQ175) show that phagocytic microglia are enriched in disease affected regions (striatum and motor cortex) and engulf cortico-striatal synapses prior to the appearance of motor and cognitive deficits. Super-resolution microscopy reveals that complement proteins localize to vulnerable cortico-striatal synapses during periods of active synapse loss and treatment with a novel C1q blocking antibody is able to reduce synapse loss in these HD models. Together, these results suggest that aberrant interactions between the complement system and microglia may drive early loss of cortico-striatal synapses and contribute to behavioral deficits and neurodegenerative pathology in HD.

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GROUP A: HD BIOLOGICAL MECHANISMS

Activation of TrkB receptor by a novel monoclonal antibody agonist: Implications for the treatment of Huntington's disease**Ewa Rollmann**

Evotec

Huntington's disease (HD) is a debilitating genetic neurodegenerative disease caused by a trinucleotide repeat expansion in the huntingtin gene (HTT) that leads to production of a pathogenic mutant huntingtin protein (mHTT). Recent evidence suggests that alterations in the neurotrophin tyrosine kinase receptor signaling pathways also contribute to HD pathophysiology. Brain-derived neurotrophic factor (BDNF)-mediated activation of the tyrosine kinase B (TrkB) receptor is a key pathway for the survival, differentiation and synaptic plasticity of striatal neurons. Reduced levels of BDNF have been previously reported in both HD post-mortem brain tissue and HD mouse models. Furthermore, the presence of mHTT is associated with reduced levels of BDNF in the striatum, likely arising from impaired gene expression and cortico-striatal trafficking, and altered TrkB signaling. While BDNF has been shown to have neuroprotective effects in the striatum, increased TrkB activation in the hippocampus has been associated with seizure activity. In order to validate potential therapeutic approaches in pre-clinical HD models, we explore the ability of a novel mouse TrkB agonistic monoclonal antibody (mAb TrkB agonist from Pfizer) to activate the TrkB receptor signaling pathway *in vivo*. The mAb TrkB agonist was initially tested *in vitro* to confirm its functional activity. Subsequently, wild-type mice received intrastriatal bolus injections of mAb TrkB agonist at 6 weeks of age and were sacrificed at multiple time-points post-injection. At 30 min post-injection, a significant increase in the phosphorylation of TrkB was observed in striatal neurons of mice treated with mAb TrkB agonist when compared to vehicle-treated and non-treated animals. At 4 hours post-injection, the levels of TrkB phosphorylation were back to control levels. As a complementary approach, we also examined the effects of BDNF gene delivery *in vivo*. WT mice received a single unilateral striatal injection of AAV-proBDNF-HA and were sacrificed 6 weeks post-injection. Unexpectedly, an unusual high mortality rate preceded by seizures was observed exclusively in AAV-proBDNF-HA treated mice around three weeks after treatment was initiated. Upon histological analysis of the injected brains, significant HA expression was detected in hippocampal neurons of affected mice. After adjustment of injection coordinates to prevent AAV-distribution to the hippocampus, all mice survived the treatment and results from this study will be presented here. Overall, our findings demonstrate the functional activity of a mAb TrkB agonist antibody in the CNS, and support an immunotherapeutic approach to restore or prevent aberrant BDNF-TrkB signaling activity in the HD brain.

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GROUP A: HD BIOLOGICAL MECHANISMS

Mutant huntingtin disrupts the nuclear pore complex**Jonathan C. Grima**

Johns Hopkins University

Huntington's disease (HD) is a hereditary and incurable neurodegenerative disorder caused by an expanded CAG repeat in the first exon of the huntingtin (*htt*) gene, resulting in progressive degeneration of striatal medium spiny neurons. Disease onset and severity are dependent on CAG repeat length with a longer expansion resulting in earlier onset and greater severity. However, the underlying mechanisms by which mutant *htt* causes the disease have not been fully elucidated. Nonetheless, some studies suggest that nucleocytoplasmic transport dysfunction could be a key pathogenic contributor. The trafficking of RNA and proteins between the cytoplasm and the nucleus is a critical aspect of signal transduction and is especially arduous for neurons due to their highly polarized structure. This process is tightly regulated by Nuclear Pore Complexes (NPCs), which are aqueous protein channels that span the entire nuclear envelope and serve as the main gateway between the nucleus and cytoplasm. The NPC is one of the largest protein complexes in eukaryotic cells and consists of multiple copies of approximately 30 different protein subunits called Nucleoporins (NUPs). NUPs are organized into 5 main anatomical regions of the NPC (cytoplasmic ring/filaments, central channel, transmembrane, scaffold, nuclear ring/basket) and each play unique roles in overall NPC transport function, such as mRNA export and protein import/export. Interestingly, it was recently discovered that some of the longest-lived proteins in the mammalian brain are NUPs located in the scaffold of the NPC and may represent the "weakest link" in the aging proteome. Recent live-cell imaging studies show that mutant *htt* demonstrates reduced dynamics and rates of nucleocytoplasmic transport. Cell culture and transgenic animal models display distortions in nuclear envelope and increases in the clustering of NPCs. Studies have demonstrated that mutant *htt* can preferentially bind certain components of the NPC and that cytoplasmic protein aggregates can interfere with nucleocytoplasmic transport of protein and RNA. And finally, recent studies show that RAN translation products, which have been shown to disrupt nucleocytoplasmic transport in C9orf72 ALS-FTD, are also produced in HD. We hypothesize that products of the mutant *htt* repeat expansion are likely to disrupt nucleocytoplasmic transport at the NPC. To this end, we assessed NPC integrity in various transgenic mouse tissues, iPS neurons, and postmortem human tissue. Our data indicate that select NUPs involved in nucleocytoplasmic trafficking are affected as evidenced by nuclear aggregation that co-localizes with mutant *htt*. This study suggests that the NPC is disrupted in HD.

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GROUP A: HD BIOLOGICAL MECHANISMS

Progress towards the identification of a selective MLK3 inhibitor for proof of concept studies in animal models of Huntington's disease**Thomas Krulle**

Evotec

MLK3 (mixed lineage kinase 3) is a serine/threonine protein kinase that functions as an upstream activator of the JNK (c-Jun N-terminal protein kinases) pathway. It has been proposed that an interaction between the MLK3- SH3 (SRC homology 3) domain and mutant huntingtin (mHTT) precludes MLK3 auto-inhibition and leads to a disruption of fast axonal transport (FAT) via downstream phosphorylation of kinesin motor in neurons. Activation of the JNK pathway via MLK3 is also known to induce apoptosis through c-Jun phosphorylation (1). Here we describe the identification of small molecule MLK3 inhibitors that are selective over most other relevant pathway kinases, such as TAK1, MLK2, ASK1, MKK4, MKK7, JNK1 and JNK3. Our structure based drug design (SBDD) approach was driven by several MLK3 kinase domain/inhibitor co- crystal structures solved at Evotec. Our compounds exhibited similar cellular potency to CEP-1347, a known non-selective MLK3 inhibitor (2), in a MLK3- overexpressing HEK cell assay. Our lead compounds were also able to restore FAT in a squid axoplasm perfusion assay, comparable to the effect seen with the JNK3 inhibitor SP600125 (3). We further demonstrated that these in vitro proof-of-concept compounds can be improved to achieve brain penetration and exposure, making them suitable for proof-of-concept studies in animal models of Huntington's disease. (1) C. E. Bazenet et al., Evidence for a Role of Mixed Lineage Kinases in Neuronal Apoptosis, *The Journal of Neuroscience*, 2001, 21 (14), 4949-4957 (2) B. L. Apostol et al., CEP-1347 reduces mutant huntingtin-associated neurotoxicity and restores BDNF levels in R6/2 mice, *Mol. Cell. Neurosci.*, 2008, 39 (1), 8-20 (3) G. A. Morfini et al., Pathogenic huntingtin inhibits fast axonal transport by activating JNK3 and phosphorylating kinesin, *Nat. Neurosci.*, 2009, 12, 864- 871

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GROUP A: HD BIOLOGICAL MECHANISMS

Progress towards the identification of a potent and selective ATM inhibitor suitable for a proof-of-concept study in HD models**Rebecca Jarvis**

Charles River

Emerging evidence shows that the ATM signalling pathway is dysregulated in neurodegenerative disorders including Huntington's Disease (HD). ATM signalling has been shown to be elevated in cells derived from HD patients and mouse models of HD. Genetic and pharmacological evidence from cellular and animal models of HD suggests that reduction of ATM signalling can ameliorate mHTT toxicity. Thus inhibitor of ATM kinase presents a promising therapeutic intervention strategy for the treatment of HD. We present data from our medicinal chemistry program which aims at developing potent and selective and brain-penetrant ATM inhibitors. Chemical optimization of CNS-compliant physical chemical properties led to the identification of compounds suitable for a proof-of-concept study in HD models. Our lead compound, CHDI-00485194, displayed excellent oral bioavailability and pharmacokinetics. PO administration to mice showed distribution into brain (brain:plasma ratio of 1.3) and linear pharmacokinetics in a dose escalation study. An acute PK/PD biomarker study to evaluate the effect of CHDI-00485194 on irradiation induced DNA damage biomarkers is ongoing.

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GROUP A: HD BIOLOGICAL MECHANISMS

Phospho-proteomic analysis of zQ175 mice with HDAC4 genetic knockdown reveals nuclear and cytoplasmic regulated proteins that may contribute to HD phenotype improvement**Christoph Schaab**
Evotec

Histone deacetylase 4 (HDAC4) belongs to the Class IIa HDAC family of transcriptional repressors. HDAC4 reduction improved disease-related phenotypes in the R6/2 and HdhQ150 HD models (Mielcarek et al. 2013) and synaptic, neuronal and behavioral improvements were seen in the zQ175 heterozygous (zQ175 HET) knock-in mouse model with a heterozygous HDAC4 deletion (HDAC4 KD). These beneficial effects occurred without significant effect on reversal of mHtt-induced global transcriptional dysregulation, raising mechanistic queries on how HDAC4 KD impacts HD pathology independently of a role in transcriptional regulation. We used quantitative mass spectrometry to analyze the phospho-proteome of striatal samples from 13 month zQ175 HET mice with and without HDAC4 KD. HDAC4 KD had a moderate impact on the global phosphoproteome, showing 61 significantly regulated phosphorylation sites. Prominent phosphorylation changes were observed with NMDA receptor signaling, including decreased phosphorylation on the downstream effector Camk2g. Aberrant NMDA receptor activation is associated with the HD neurodegenerative phenotype (Fan et al. 2007). Our findings indicate a modification of NMDA signaling resulting from HDAC4 KD, consistent with reduced calcium influx and potential modification of the signaling processes controlling HDAC4 nuclear-cytoplasmic shuttling. Mapping the phosphoproteomic differences onto STRING protein-protein interaction networks additionally identified other significantly regulated subnetworks, including modules of gene expression regulators, proteins regulating the splicing machinery and a nuclear subnetwork of histones and histone modifiers. Global proteomics analysis showed 74 significantly altered proteins associated with HDAC4 KD in Q175 mice. Noticeable downregulation in metabolic enzymes such as Pdk2 and Pdpr suggest altered oxidative phosphorylation and carbohydrate metabolism. Upregulation of PGC-1 α was also observed; transcriptional interference of this gene has previously been implicated in HD pathology (Tsunemi et al. 2012). Lastly, the restored synaptic function observed in these mice may be related to expression changes in WAVE regulatory complex (WRC) proteins, critical elements for neurite development and proper brain connectivity, as well as the differential regulation of other cytoplasmic proteins associated with regulation of actin dynamics and dendritic morphogenesis. In summary, our phospho-proteomics analyses of brain tissue from zQ175 HET mice with HDAC4 KD provides further exploratory avenues to pursue mechanisms by which reduction of HDAC4 in the CNS is beneficial in HD models.

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GROUP A: HD BIOLOGICAL MECHANISMS

Ablation of p62 modulates levels of soluble and aggregated mutant huntingtin and delays end-stage disease in R6/2 mice**Gillian P. Bates**
King's College London

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by the dominantly inherited expansion of a CAG repeat within the coding region of the HD gene (HTT). N-terminal fragments of the mutant huntingtin (HTT) protein aggregates into oligomeric and fibrillary structures that can be detected as polyubiquitylated inclusion bodies in tissue sections. Misfolded and aggregated proteins are degraded through two main intracellular protein clearance systems, the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway. p62 is an adapter protein that regulates the proteolysis of ubiquitylated proteins via selective autophagy. We show that in both the R6/2 and HdhQ150 knock-in mice, p62 relocates to the nucleus with disease progression where it forms high molecular weight structures and co-localizes with HTT inclusions. To assess the effect of p62 ablation in R6/2 mice, we bred R6/2 males that were heterozygous for p62 knock-out (p62Het) to p62Het females to generate WT, p62Het, p62 homozygous knock-out (p62Hom); R6/2, R6/2::p62Het and R6/2::p62Hom progeny. We have been able to demonstrate by multiple biochemical analyses that the levels of soluble and aggregated HTT are decreased in multiple brain regions of R6/2::p62Hom mice, as compared to their R6/2 littermates and that this effect becomes more pronounced with disease progression. These data are complemented by the quantitative immunohistochemical analyses presented on the poster by Tillack et al. Ablation of p62 in R6/2 mice did not modify the failure of R6/2 mice to gain body weight, or impairments in rotarod performance, grip strength or activity but did delay end-stage disease as previously reported (Kurosawa et al. 2014). Similarly, ablation of p62 did not improve dysregulated transcriptional profiles. Unravelling the mechanism by which the manipulation of p62 modulates soluble and aggregated HTT levels will lead to a greater understanding of the aggregation and clearance of mutant HTT. Kurosawa et al. (2015) Hum Mol Genet 24:1092-105.

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GROUP A: HD BIOLOGICAL MECHANISMS

A homeostatic brain model of plasticity and risk in Huntington's disease**James Kozloski**
IBM Research

Recently, IBM Research has initiated a brain modeling effort around Huntington's disease in collaboration with CHDI. Here we describe a key driver of this project: a closed loop model of resting state activity in the brain that invokes synaptic plasticity at specific neural tissue interfaces to achieve certain system set points. This model of the cortico-striato-pallido-thalamo-cortical system is now implemented in conjunction with IBM's Neural Tissue Simulator, allowing 1) elaboration of this network and its component tissues with ever greater biological realism and complexity, and 2) prediction of biophysical changes to different tissues given changes to system set points. We describe applications of the model and our simulation capabilities to the study of neurodegenerative diseases including Huntington's, wherein disorder is modeled first as a disturbance in plasticity at tissue interfaces such as the corticostriatal synapse, and then quantitatively analyzed for increased risk of neuronal death and system dysfunction. Elaboration of the simulation within the model's different tissues then serves as a test bed for in silico rendering of therapeutic targets and interventions.

Pengsheng Zheng, Tuan Hoang Trong, Tim Rumbell IBM Research

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GROUP A: HD BIOLOGICAL MECHANISMS

Modulation of p62 in zQ175 and R6/2 mice promotes mutant huntingtin clearance**Karsten Tillack**
Evotec

Selective autophagy is a catabolic biological process for the removal of polyubiquitinated proteins. It plays a role in clearance of aggregated proteins that are not degraded by the proteasome such as mutant huntingtin (mHTT). Targeting key modulators of autophagy has become an attractive new approach in the development of mHTT lowering therapeutics for Huntington's disease (HD). p62 is an autophagic adaptor molecule that facilitates sequestration of aggregated protein cargo into the autophagosome via interaction with LC3. Previous reports indicate that p62 is co-localized with nuclear mHTT inclusions in both HD mouse models and post-mortem HD brain tissue. In the present study we investigated whether modulation of p62 protein expression would influence mHTT aggregation in HD mouse models using different time-points and approaches to interfere with p62 expression. We employed AAV-mediated delivery of p62-specific shRNA into the striatum of zQ175 mice, followed by quantification of mHTT inclusion level by high content histology. In young zQ175 mice, knockdown (KD) of p62 prior to manifestation of mHTT inclusions led to a significant increase in extra-nuclear mHTT inclusions, as well as significant reduction in the size and number of nuclear inclusions. In contrast, when p62 KD was performed at an age when mHTT inclusions were already present, the subsequent shift between nuclear and extra-nuclear inclusions was attenuated. We also quantified mHTT inclusion levels in the cortex and striatum of R6/2 mice with p62 genetic deletion at 4, 9 and 14 weeks. While a reduction of nuclear inclusion number and size in 9 and 14 week R6/2 mice with p62 deletion could be detected, the effect was only significant in the striatum and much less pronounced in the cortical region. In addition, the change in extra-nuclear inclusions was smaller in R6/2 mice with genetic deletion of p62 than in zQ175 with p62 KD. These findings add further evidence that p62 has a role in regulating degradation of mHTT. Additional studies are crucial to understand these mechanisms and optimize mHTT clearance strategies.

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GROUP A: HD BIOLOGICAL MECHANISMS

The HTT CAG expansion mutation determines age at death but not disease duration in Huntington's disease**Jong-Min Lee**

Massachusetts General Hospital

Huntington's disease (HD) is caused by an expanded HTT CAG repeat that leads in a length-dependent, completely dominant manner to onset of a characteristic movement disorder. HD also displays early mortality, so we tested whether the expanded CAG repeat exerts a dominant influence on age at death and on the duration of clinical disease. We found that, as with clinical onset, HD age at death is determined by the expanded CAG repeat length with no contribution from the normal CAG allele. Surprisingly, disease duration is independent of the mutation's length. It is also unaffected by a strong genetic modifier of HD motor onset. These findings suggest two parsimonious alternatives: 1) HD pathogenesis is driven by mutant huntingtin but, before or near motor onset, sufficient CAG-driven damage has occurred to permit CAG-independent processes to then lead to eventual death. In this scenario, some pathological changes and their clinical correlates may still worsen in a CAG-driven manner after disease onset but these CAG-related progressive changes do not themselves determine duration. Alternatively, 2) HD pathogenesis is driven by mutant huntingtin acting in a CAG-dependent manner with different time courses in multiple cell-types, and the cellular targets that lead to motor onset and to death are different and independent. In this scenario, HTT CAG length-driven processes lead directly to death but not via the striatal pathology associated with motor manifestations. Each scenario has important ramifications for the design and testing of potential therapeutics, especially those aimed at preventing or delaying characteristic motor manifestations.

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GROUP A: HD BIOLOGICAL MECHANISMS

HTT haplotype-independent CAG repeat instability**Jiwoo Lee**

Academy for Medical Science Technology

Expansion of polymorphic huntingtin (HTT) CAG trinucleotide repeat beyond 35 is responsible for essentially all cases of Huntington's disease (HD). The expanded CAG repeats are unstable and further undergo size-dependent and expansion-biased repeat instability in germ cells and brain regions that may account for genetic anticipation and accelerated pathogenesis leading to clinical manifestation of HD. Identification of factors responsible for repeat instability is important as these may serve as therapeutic targets of disease modifying interventions. Here, we determined whether HTT haplotype influences the variable levels of intergenerational repeat instability in humans. The most frequent 8 haplotypes based on common SNPs were tested for their association with 1) CAG repeat length distributions in the HD population and 2) repeat length differences within families. Expanded CAG alleles on the hap.01 haplotype, the most common HTT haplotype in HD subjects of European ancestry, showed a size distribution similar to other haplotypes. In addition, the frequencies of disease haplotypes in adult onset HD and those of juvenile onset HD were not significantly different, indicating that juvenile onset HD subjects are not enriched for a specific disease haplotype and implying that the propensity of different haplotypes toward further CAG expansion is largely similar. This proposition was further supported by observations in HD families. HD families constructed based upon genome-wide genotypes indicated that neither 1) the variance in CAG repeat sizes within families nor 2) the change of CAG repeat sizes in a parent to a child transmission were associated with HTT haplotype. However, gender and the length of repeat of a transmitting HD parent were significantly associated with changes in the allele transmitted to an HD child; HD fathers with long CAG repeats tended to generate children with further expanded CAG repeats. Taken together, these findings suggest that processing of CAG repeats in the germ cell is not different between haplotypes, but is dependent on gender and on the size of repeat of a transmitting parent. Relatively stable transmissions from female HD parents with long CAG repeats also raise the possibility of factors that suppress CAG repeat expansion whose delineation could provide interventional strategies to prevent genetic anticipation through the male germline.

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GROUP A: HD BIOLOGICAL MECHANISMS

A molecular basis for sarcopenia in human Huntington's disease**Patrick Weydt**
Ulm University

Metabolic problems such as emaciation and muscle wasting are prominent aspects of advanced stages of Huntington's disease (HD) and a significant cause of morbidity. Even though these features are recapitulated in many transgenic mouse models of the disease, the underlying molecular mechanisms remain incompletely understood, severely limiting the options for rational therapeutic interventions. PGC-1 α dysregulation in the CNS and peripheral tissues is a hallmark of HD and possibly contributes to the severe sarcopenia and cachexia in advanced stages. PGC-1 α expression and activity in muscle is induced by exercise thus we speculated that RNA polymerase I transcriptional activity should also be activated by physical exercise. 21 untrained healthy persons of age 18-65 performed a 30 minutes exercise ergometer training and muscle biopsies were taken before and 3h after exercise. Additionally, we performed muscle biopsies before and 3h after exercise in 7 gene carriers of the Huntington's disease mutation. Total RNA was harvested and PGC-1 α expression and RNA polymerase I activity were detected by qPCR. Exercise significantly induced PGC-1 α mRNA expression and stimulated RNA polymerase I transcription in healthy donors. Although we detected an increase PGC-1 α expression and 47S rRNA transcription after exercise in patients with early stages of Huntington's disease, the overall transcriptional activity of RNA polymerase I in muscle tissues was severely compromised. This result extends the hypothesis, that PGC-1 α regulates rDNA transcription under different stress conditions to the observation that even basal levels of ribosomal biogenesis seem to be affected by dysregulation of PGC-1 α in Huntington's disease.

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GROUP A: HD BIOLOGICAL MECHANISMS

Examining the role of DAPK1 in mediating extrasynaptic NMDA receptor excitotoxicity in Huntington disease**Mandi E Schmidt**
University of British Columbia

Huntington disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in exon 1 of the huntingtin (HTT) gene. HD is characterized by corticostriatal synaptic dysfunction and progressive neuronal death in the striatum, leading to motor, psychiatric and cognitive disturbances. In multiple HD mouse models including the YAC128 transgenic model expressing human mutant HTT with 128 CAG repeats, one of the earliest synaptic changes to occur is enhanced striatal extrasynaptic N-methyl-D-aspartate receptor (exNMDAR) expression and activity. This activity is mediated primarily through GluN2B subunit-containing receptors and is associated with increased cell death signaling and NMDA sensitivity in YAC128 striatal medium spiny neurons, the most vulnerable neuronal population in HD. Death-associated protein kinase 1 (DAPK1) is a pro-apoptotic calcium/calmodulin-regulated serine/threonine protein kinase highly expressed in neurons. Although DAPK1 is believed to play a major role in neuronal death during development, its expression and activation are rapidly increased in vivo in response to ischemia. Furthermore, under excitotoxic conditions, DAPK1 is recruited to extrasynaptic GluN2B-containing complexes and phosphorylates the receptor, increasing its conductance. DAPK1 is thus proposed to act as an exNMDAR signal amplifier. We provide evidence to suggest that DAPK1 may contribute to early exNMDAR excitotoxicity in the YAC128 mouse model of HD. Our new data indicates that DAPK1 may be a novel target for HD therapies.

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GROUP A: HD BIOLOGICAL MECHANISMS

RAN translation in Huntington disease

Monica Banez-Coronel

University of Florida

Huntington disease (HD) is a neurodegenerative disorder characterized by adult-onset of motor dysfunctions and psychiatric disturbances, which is caused by a CAG•CTG expansion in the huntingtin (HTT) gene. While most research has focused on the HTT polyGln-expansion protein, we demonstrate that four additional, novel, homopolymeric expansion proteins (polyAla, polySer, polyLeu, polyCys) accumulate in HD human brains. These sense and antisense repeat-associated non-ATG (RAN) translation proteins accumulate most abundantly in brain regions with neuronal loss, microglial activation and apoptosis, including the caudate/putamen, white matter regions, and in juvenile-onset cases, also the cerebellum. RAN protein accumulation and aggregation are length-dependent and individual RAN proteins are toxic to neural cells independent of RNA effects. These data suggest RAN proteins contribute to HD, and that therapeutic strategies targeting both sense and antisense genes may be required for efficacy in HD patients. This is the first demonstration that RAN proteins are expressed across an expansion located in an open-reading-frame and suggests RAN translation may also contribute to other polyglutamine diseases.

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GROUP A: HD BIOLOGICAL MECHANISMS

Defining a therapeutic regimen for the delivery of cholesterol to Huntington disease neurons

Eleonora Di Paolo

University of Milan

There is solid evidence showing reduced brain cholesterol biosynthesis in HD. Isotopic dilution mass spectrometry (MS) in combination with biochemical and molecular analyses, show that cholesterol precursors are already reduced before the onset of symptoms in the brain of five HD rodent models and that cholesterol content is significantly decreased at later time points. These data were recently confirmed on brain samples from Q175 mice in a cross-validation study involving two laboratories (Shankaran, et al. and Valenza, submitted). We found consistent reduction in daily synthesis rate of cholesterol (by 2H₂O labeling), reduced lathosterol, starting from pre-symptomatic stages, as well as cholesterol at later stages (Shankaran, et al. and Valenza, submitted). In vitro, cholesterol dysregulation emerges in HD astrocytes and is linked to reduced activity of the cholesterologenic SREBP transcription factor (Valenza, Cell Death and Differentiation 2015). Abnormalities in cholesterol biosynthesis, is paralleled by reduced plasma level of 24-OHC, a specific catabolite of brain cholesterol that is able to cross the BBB, in HD patients (Leoni, Brain 2008). As cholesterol is implicated in multiple neuronal functions, a decrease in local cholesterol biosynthesis may be detrimental. Recently, we tested whether we can rescue aspects of neuronal dysfunction in vivo by supplying exogenous cholesterol directly in the brain. In our first study, conducted with colleagues from University of Modena, Besta Neurological Institute and UCLA, we found that by delivering an estimated dose of 20ug of cholesterol via brain-permeable polymeric nanoparticles (g7-NPS-Chol) cognitive defects are rescued and there is a partial amelioration of global motor activity in R6/2 mice (Valenza, Embo Mol.Medicine 2015). In a second unpublished and ongoing study, R6/2 mice were intrastrially infused with cholesterol, at a continuous rate, via osmotic minipumps. In the single MS measurement so far conducted we were able to detect exogenous cholesterol in striatum of wt mice. Furthermore, infusion of 20ug cholesterol in R6/2 mice (and controls, N=10/group) showed improved cognitive function. More recently, we have joined forces with Mario Negri Institute in Milano in order to test a third strategy based on intranasal delivery of cholesterol formulations. Preliminary results will be presented. Hopefully, these studies will be continued in the context of a recently submitted proposal aiming at providing long-term supplementation of the therapeutic dose of cholesterol to HD neurons via the most efficacious route and followed by an in depth whole-animal assessment of molecular, functional and behavioral parameters.

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GROUP A: HD BIOLOGICAL MECHANISMS

Machine learning for neuroimaging analytics and imaging-based biomarkers**Guillermo A. Cecchi**

IBM Research

To date neuroimaging based measures, in particular volumetric change, have shown the greatest promise in reaching effect sizes relevant to evaluate response to potential therapeutic interventions in HD. Functional imaging is less developed as a potential biomarker tool but provides highly complex feature sets that appear connected to compensatory changes in early disease. We have recently initiated a collaboration with CHDI with the goal of deploying multi-modal neuroimaging data, synergy of multiple effects and cross modal interactions to further characterize mechanistic disease progression and to identify significant feature changes that singly or in combination may provide sensitive candidate biomarkers to evaluate treatment effects of future therapeutic interventions. We will present the approach and scope of this collaboration in order to find possible synergies with other HD researchers. Specifically, we plan to identify relevant imaging-based markers in HD through the use of high-dimensional imaging data, including sMRI, DTI and fMRI. The analytic approach will be based on theoretical models with mechanistic interpretation, and will rely heavily on our expertise in multivariate and state-of-the-art statistics and machine learning techniques applied to neuroimaging. In addition, the parties will test mechanistic hypotheses such as anatomical connection-based, activity-based, and correlation or network-based models of disease progression. We will thoroughly exploit the available complexity of neuroimaging features and associations with clinical parameters of change, and recent developments in machine learning including Deep Learning for structural and functional data, regularization for Markov Networks models of multi-variate interactions, causal modeling for functional and longitudinal data, voxel-based and non-linear dynamics-based functional network models. The outcome is expected to develop novel neuroimaging biomarkers of clinical relevance and in combination, to improve the sensitivity and precision of existing metrics. The utility of promising candidate biomarkers can be tested against future data sets and by using new experimental designs.

Eduardo Castro, Pablo Polosecki and Irina Rish. IBM Research



[GROUP B]

Poster Abstracts

HD Models

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GROUP B: HD MODELS**Increased P-glycoprotein expression in brain capillaries of Huntington's disease transgenic mice and patients.****Yu-Han Kao**

National Taiwan University

Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by chorea, dementia, and psychiatric symptoms. Although treatments to alter the course of HD are to be developed, combinatorial drug therapy is prescribed for symptom control of HD. Given the diversity of drugs used in the treatment of HD, the changes in the pharmacokinetics, including absorption, the transfer across the blood-brain barrier (BBB), and elimination of drug substances are worth an attention. As transporters are important for the pharmacokinetics of drugs, in this study, we compared the mRNA levels of transporters in brain cortex, intestine, liver, and kidney of R6/2 HD transgenic mice to those in littermate control mice. The results showed that *mdrla* mRNA (encoded P-glycoprotein) was significantly higher in all these tissues of R6/2 HD mice than in controls. Consistent with this increase of mRNA level in the brain of R6/2 mice, higher protein expression of P-glycoprotein was also observed in brain capillaries of both R6/2 mice and human HD patients. As P-glycoprotein is an efflux transporter that can significantly reduce the entry of its substrates across the BBB, we further investigated the impacts of the increased P-glycoprotein on brain concentrations of antipsychotic agents risperidone and paliperidone following intraperitoneal administrations using an in-vivo brain microdialysis technique. As a result, brain extracellular levels of risperidone and paliperidone were significantly lower in R6/2 mice than in their littermate controls. Furthermore, the concomitant use of tariquidar, a P-glycoprotein inhibitor, can inhibit the efflux activity of P-glycoprotein at the BBB and increase extracellular levels of risperidone in the brain. These findings suggest that the pathology state of HD may regulate the expression of P-glycoprotein and markedly affected the availability of P-glycoprotein substrates in the brain. Further studies are required to verify clinical significance of these findings.

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Hui-Ting Yang, School of Pharmacy, National Taiwan University Hui-Mei Chen, Institute of Biomedical Sciences, Academia Sinica Chun-Jung Lin, School of Pharmacy, National Taiwan University

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GROUP B: HD MODELS**Evaluation of new behavioral readouts on Huntington's disease in the BACHD rat****Tiberiu Loredan Stan**

QPS Austria

Huntington's disease (HD) is an inherited, autosomal dominant neurodegenerative disease. Symptoms of HD patients include motor, emotional and cognitive deficits. Apart from impairments of motor coordination swallowing problems are common among people with HD. At the present, there is no cure for HD and animal models are considered a valid option in order to gather novel insights from both molecular and behavioral level. BACHD rats, one of the latest animal models for HD, overexpress the full-length human mutant huntingtin gene with 97 alternating CAA/CAG repeats under control of the human HD promoter. Animals develop behavioral and neuropathological abnormalities reminiscent of clinical signs in HD patients. In this study we used wild type and transgenic rats of both genders at the age of 12-15 months. We investigated BACHD rats regarding nesting behavior and pasta gnawing. Nesting behavior involved a quantitative measurement of the nesting material that had been placed in the food grid and had to be actively pulled out into the cage to build a nest over 24 h. In the pasta gnawing test male rats received pieces of uncooked spaghetti in their keeping cage and while eating, gnawing noises of the animals were recorded in a soundproof cabinet. Animals also performed the novelty-induced hypophagia (NIH) test, a behavioral paradigm sensitive to anxiety and depression-like states. BACHD females used a decreased amount of nesting material as compared with wild type littermates, while BACHD males did not differ from wild type rats in this test. In the pasta gnawing test, BACHD males showed a decrease of the number of bites per selected chewing episodes as compared with wild type animals. In the NIH test, BACHD males showed a decreased latency to drink sweetened milk in the keeping cage when compared with wild type animals. Both genotypes had an increased latency to drink milk in a novel cage without bedding and under bright light as compared to the home cage situation, whereas no genotype differences were found for this parameter. Altogether, our data suggest that nest-building behavior in female rats and pasta-gnawing behavior in male rats are interesting parameters to be further explored as readouts for treatment studies.

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GROUP B: HD MODELS

An enhanced Q175 knock-in mouse model of Huntington disease**Amber Southwell**
University of British Columbia

Huntington disease (HD) model mice with heterozygous knock-in (KI) of an expanded CAG tract in exon 1 of the mouse huntingtin (Htt) gene homolog closely genetically recapitulate the mutation that causes HD, and might be favored for preclinical studies. However, historically these mice have failed to phenotypically recapitulate the human disease. Thus, homozygous KI mice, which lack wildtype Htt, and are much less relevant to human HD, have been used. The zQ175 model is the first KI mouse to exhibit significant HD-like phenotypes when heterozygous. In an effort to exacerbate HD-like phenotypes and enhance preclinical utility, we backcrossed zQ175 mice to FVB/N, a strain highly susceptible to neurodegeneration. These Q175F mice display significant HD-like phenotypes along with sudden early death from fatal seizures. The zQ175 KI allele retains a floxed neomycin resistance cassette upstream of the Htt gene locus and produces dramatically reduced mutant Htt as compared to the endogenous wildtype Htt allele. By intercrossing with mice expressing cre in germ line cells, we have excised the neo cassette from Q175F mice generating a new line, Q175F neo (Q175FDN). Removal of the neo cassette resulted in a ~2 fold increase in mutant Htt and rescue of fatal seizures, indicating that the early death phenotype of Q175F mice is caused by Htt deficiency rather than by mutant Htt. Additionally, Q175FDN mice exhibit earlier onset and a greater variety and severity of HD-like phenotypes than Q175F mice or any previously reported KI HD mouse model, making them extremely valuable for preclinical studies.

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GROUP B: HD MODELS

Browning of white adipose tissue in the R6/2 mouse model of Huntington's disease**Maria Björkqvist**
Lund University

In both human HD and mouse models of HD, there is evidence of increased energy expenditure and weight loss, alongside altered body composition including adipose tissue abnormalities. The huntingtin gene is expressed through out the body and accumulating evidence indicates that a direct effect of mutant huntingtin, within peripheral cells and tissues, occurs independently from neurological defects as well as contributes to HD pathology. Recent mouse studies suggest that HD central pathology can be ameliorated by targeting peripheral manifestations and illustrates the importance of acquiring knowledge on peripheral pathology in HD. In this study, we therefore investigated the gene expression profile, histological appearance and functional aspects of white adipose tissue (WAT) in the R6/2 mouse model of HD. Besides classical brown and white adipocytes, knowledge have accumulated over the last decade describing a sub-set of cells within WAT that can be converted to "beige" or "brite" or "brown-in-white" adipocytes, sharing many of the morphological and functional features of brown adipocytes. These cells can upon stimulation, by for example cold exposure and certain transcription factors, adopt brown-like features, such as UCP1 gene expression. WAT from R6/2 mice contained significantly more brown adipocyte-like regions than that of wild-type (WT) mice and had a gene expression profile suggestive of the presence of brown-like adipocytes, such as higher UCP1 expression. Cold exposure induced UCP1 expression in R6/2 WAT to a markedly higher degree as compared to the thermogenic response in WAT of WT mice. Alongside this, gene expression of the transcription factors CREB1 and PPARα, important inducers of WAT browning, were increased. We could also confirm previous data, showing a blunted UCP1 expression response in R6/2 BAT, highlighting the complexity of adipose tissue in thermogenesis. While the genetic profile of R6/2 WAT suggests altered lipolytic function, we did not observe altered WAT lipolytic capacity of R6/2 mice at 12 weeks of age. Our results suggest browning of WAT in R6/2 mice, which could potentially contribute to R6/2 weight loss. This work was funded by the Swedish Research Council and the Royal Physiographic Society of Lund.

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GROUP B: HD MODELS

Altered dopamine receptor 2 signaling in the zQ175 mouse model of Huntington's disease.**Ladislav Mrzljak**

CHDI Foundation/CHDI Management

Huntington's disease (HD) is a genetically inherited neurodegenerative disorder characterized by severe motor dysfunction, cognitive decline and psychiatric disturbances, associated with profound striatal and cortical loss. Altered dopaminergic transmission is thought to play a key role in HD pathophysiology, and has been reported in HD patients as well as in rapidly-progressing rodent models of HD. To enhance our understanding of such differences, we aimed to characterize dopamine dynamics in the slower-progressing knock-in zQ175 mouse model of HD. Electrically-evoked striatal dopamine release from anesthetized 7 month old heterozygous zQ175 and WT mice was detected by fast-scan cyclic voltammetry. Oxidative currents were measured in the ventral striatum while increasing intensities of electrical stimulation were delivered to the VTA/SNc after vehicle and after raclopride (D2 antagonist; 1 mg/kg) i.p. injection. Dopamine levels detected from WT and Q175 mice after vehicle treatment were not significantly different. However, raclopride-evoked enhancement of phasic dopamine release was attenuated in Q175 animals as compared with WT. There was no difference in the rate of reuptake between genotypes as measured by Tau75. These results indicate presynaptic D2 receptor dysfunction in Q175 mice, and suggest that similar functional alterations may exist at the postsynaptic level, impairing striato-pallidal indirect pathway transmission.

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GROUP B: HD MODELS

Phenotype development in F2 generation of TgHD minipigs followed by invasive approaches: mtHTT fragmentation and proteolytic enzymes in brains**Zdenka Ellederova**

Institute of Animal Physiology and Genetics

The Huntington's disease (HD) is associated with neuronal death and the formation of aggregates in basal ganglia and cerebral cortex. Nevertheless, previous studies showed that even neurons without aggregates undergo degeneration and cell death. Furthermore, proteolytic fragments derived from mutant huntingtin (mtHtt) were found to be toxic to neurons, which are located in the striatum and cortex. Consequently, inhibition of proteolysis of mtHtt significantly reduces neurotoxicity, and proteolytic enzymes are increasingly expressed in affected tissues. Therefore, proteolytic cleavage as a source of these breakdown products is considered as an early or even an initial step in HD pathogenesis. In order to follow the phenotype of the transgenic minipigs expressing N-terminal part of human huntingtin (TgHD, 548amino acids HTT-124CAG), we compared TgHD and wild type (WT) siblings of F2 generation at the age of 24, 36, and 48 months old with the main focus on brain. Immunohistochemical analysis could detect just a few spots resembling aggregates in coronal sections of transgenic minipigs, and none in WT. However, the filter retardation assay showed retention of higher molecular weight Htt polymeric structures in TgHD brains. We also focused on the presence of N-terminal Htt fragments. We screened several tissues for the presence of fragmentation, and found fragments in the brain, and testes of TgHD minipigs, but not significantly in other tissues. Just at the age of 48 months, we have started to see some Htt fragments in skeletal muscles. Using immunohistochemistry and Western blotting, we demonstrated significantly increased expression of protease caspase-3 in nucleus caudatus and cortex area of TgHD minipigs in comparison to WT animals. Also metalloproteinase MMP-10 showed increased expression in the caudate nucleus of TgHD minipig, but not in cortex. The higher levels of proteolytic enzymes detected in TgHD minipigs could be the cause of the increase production of mtHtt fragments in brain and thus contribute to the disease development. Support: CHDI Foundation (A-8248, A-5378), EXAM (CZ.1.05/2.1.00/03.0124), 7F14308, and RVO: 67985904, National Sustainability Programme (LO1609)

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GROUP B: HD MODELS

Phenotype progression in TgHD minipigs: Behavioral and motoric impairments in F0 and F1 generations coincide with alterations in the bioenergetic status of mitochondria in heart and muscles

Zdenka Ellederova

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In order to facilitate studies of pathogenesis and therapy of Huntington's disease (HD), we have generated transgenic minipigs (TgHD) using microinjection of a lentiviral vector encoding N-terminal (548 aa) part of human huntingtin (Htt) containing 126 CAG/CAA repeats under the control of the human Htt promoter. F0 generation of TgHD minipigs was born in July 2009, and F1 generation was born in April 2010. Around 180 animals in four generations are currently available. The aim of the study has been to follow the phenotype development, comparing TgHD minipigs and their wild type (WT) siblings. We performed a variety of behavioral tests, including tunnel test, hurdle, seesaw, skittles, cover pan, crossing a stepper, and elevated balance beam. We observed higher fear or inability to perform certain tests, delayed learning period mainly in situations inducing stress in our oldest TgHD animals (F0, F1 generations). The significant difference between WT and TgHD minipigs was noticed in the ability to step on and to cross the elevated balance beam. Wobbly movements of back legs were observed in the five years old animals. Also the weight of all the oldest animals dropped in 10% in the last year. These findings correspond with mitochondrial dysfunctions in muscles and heart. Respiratory chain complexes (RCC), Krebs cycle enzymes, pyruvate dehydrogenase (PDH) activity and amount were analyzed by spectrophotometric, radioisotope and immunoelectrophoretic methods. Respiration was measured by polarography. Electron microscopy was performed on heart and skeletal muscle sections. Although respiration of skeletal muscle mitochondria from 36-month-old TgHD 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 TgHD was detected in comparison with WT. In conclusion, the minipig model starts to show a motoric impairment phenotype, and thus to be a promising model for translational research and preclinical testing of HD. Support: CHDI Foundation (A-8248, A-5378), EXAM (CZ.1.05/2.1.00/03.0124), 7F14308, and RVO: 67985904, National Sustainability Programme (LO1609)

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GROUP B: HD MODELS

Autoradiography and branched DNA approaches to the characterization of Huntington's disease mouse model, zQ175KI

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Huntington's disease (HD) is a neurodegenerative genetic disorder that affects the brain, muscle coordination and leads to cognitive decline and psychiatric problems. Early damage is most evident in the striatum, but as the disease progresses, other areas of the brain are also conspicuously affected. In HD patients several changes in neurotransmitter receptors, like glutamate, dopamine, GABA, muscarinic, cholinergic and adenosine receptors have been reported. In the current studies, we compared receptor densities of dopamine 1 (D1R), dopamine 2 (D2R), cannabinoid 1 (CB1) and gamma-aminobutyric acid (GABA) in Huntington's disease (HD) mouse model using wild type (WT) and Q175 KI heterozygote (HET) mice at the age of 9 mo. Further, branched DNA (bDNA) analysis was performed to evaluate expression levels of dopaminergic (Drd1, Drd2) and cannabinoid CB1 (Cnr1) receptors and phosphodiesterase 10A (Pde10A) in striatum and cortex of zQ175 mice. For receptor density analysis, brain of 9 mo zQ175KI mice were coronally sectioned and saturation point of corresponding tritiated ligand to D2R (3H- raclopride), D1R (3H-SCH-23390), CB1 (3H-MePPEP) and GABA (3H- flumazenil) was analyzed using autoradiography. Specific binding of each ligand at studied region was defined by the difference between total and non- specific binding. Values for Bmax were determined using non-linear regression analysis. Significant (p<0.05) changes in Bmax of HET compared to WT were seen with all studied targets and in various brain regions. In striatum, significant decrease in D2R (32 %) and D1R (33 %) was observed. Significant decrease in Bmax in CB1 receptors was observed in HET compared to WT in globus pallidus, substantia nigra and striatum. Significant increase in Bmax in GABA receptors was seen in HET compared to WT in globus pallidus, substantia nigra, striatum and cingulate cortex. The bDNA technology revealed changes in the gene expression levels of Pde10a, Drd2, and Cnr1 in the 9 mo old HET zQ175KI male mice when compared to WT mice. Target genes were decreased in HET zQ175KI male mice to 61 %, 67 % and 32 % of WT gene expression levels for Pde10a, Drd2 and Cnr1, respectively (p<0.05). The level of Drd1 expression was also found decreased in the HET zQ175KI mice when compared to corresponding WT mice. As a summary, autoradiography and bDNA analysis are powerful research tools in preclinical Huntington's disease, and when used in combination they allow comprehensive evaluation of disease progression and treatment interventions in samples obtained from in vivo studies.

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GROUP B: HD MODELS

Fine motor kinematic analysis in R6/2 mouse model of Huntington's disease: capturing dyskinesia-like movement as a component of progressive motor deficits**Taneli Heikkinen**
Charles River

The R6/2 transgenic mice have been shown to display a progressive neurodegenerative phenotype that mimics many of the core symptoms of Huntington's disease (HD). In addition, we have previously shown characteristics and progression of fine motor deficits and gait properties of R6/2 mice. In this study, we broadened the high precision kinematic analysis to evaluate even more subtle details of movement. R6/2 and wild type mice were evaluated in two modes of ambulation: walking and wading in shallow water. Their gait was recorded with a high-speed camera as the mice traversed a 1.5 meters corridor. The movement was imaged simultaneously from three spatial dimensions, and analyzed with kinematic algorithms. In previous studies we have employed these algorithms to provide a detailed analysis of the movement profile of mice. Each point of movement trajectory was calculated as a change in coordinates and used for data analysis. The parameters analyzed, including gait characteristics and fine motor movements, were compared to wild types to evaluate specific defects. The new enhanced assessment method was able to differentiate subtle yet very significant changes in the trajectories of paw during the swing phase of stride. Interestingly, these changes could be classified as features of a dyskinesia-like movement. These specific deficits were observed during walking as well as wading which is challenging form of movement. These data provide further evidence that this kinematic fine motor analysis method captures very subtle pathological symptoms that translate to clinical features of human neurodegenerative motor diseases, and thus offers a sensitive tool to investigate the efficacy of therapeutic drugs that improve early and subtle motor functions.

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GROUP B: HD MODELS

Characterization of brain metabolic imbalance in zQ175 knock-in and R6/2 mice of Huntington's disease: principal component analysis based meta-analysis of MRS phenotypic progression**Antti Nurmi**
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In vivo 1H magnetic resonance spectroscopy (MRS) is a method used for non-invasive quantification of metabolites in various tissue types, including brain. Using MRS techniques it has been shown that in patients of Huntington's disease (HD) there are significant changes in several brain metabolites, such as N-acetylaspartate, creatine and choline. We have characterized the time course and specific features of striatal metabolic profile in R6/2 and zQ175 knock-in mice of HD, analyzing altogether 18 different metabolites. The overall metabolic changes in striatum are similar to those observed in HD patients. In this study we performed a meta-analysis of phenotypic progression of the MRS metabolic profile of the zQ175 KI and R6/2 mice. This analysis was based on large number of mice across several studies, and the analysis was implemented using a principal component analysis (PCA) based method. PCA was used to transform the total set of parameters into a new, smaller set of mutually uncorrelated parameters. In the R6/2 mice the data was obtained at 12 weeks of age, and in the heterozygous and homozygous zQ175 KI mice at various ages. The meta-analysis showed that metabolic changes occurred strikingly similarly in both examined mouse strains, with age and genotype depended progression in zQ175 KI mice. In addition, PCA revealed that there were correlations in the concentrations of several metabolites. For instance, connection in the changes of glutamine and myo-inositol appeared to be very distinctive. However, interestingly this correlation between glutamine and myo-inositol appeared to be independent of gender and genotype. Altogether, these PCA performed on MRS metabolite data provides new, broader insight in the brain metabolic profile of the zQ175 KI and R6/2 mice of HD. Moreover, this analysis method may prove to be useful and sensitive in describing pathological biomarkers that translate to clinical features of HD, as well as other human neurodegenerative diseases.

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GROUP B: HD MODELS

Behavioral phenotyping of minipigs transgenic for the Huntington gene**Ralf Reilmann**

George-Huntington-Institute

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

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GROUP B: HD MODELS

Neuroimaging of a minipig model of Huntington's disease: Feasibility of volumetric, diffusion-weighted and spectroscopic assessments**Ralf Reilmann**

George-Huntington-Institute

BACKGROUND: As novel treatment approaches for Huntington's disease (HD) evolve, the use of transgenic (tg) large animal models has been considered for preclinical safety and efficacy assessments. It is hoped that large animal models may provide higher reliability in translating preclinical findings to humans, e.g., by using similar endpoints and biomarkers. **METHOD:** We here investigated the feasibility to conduct MRI assessments in a recently developed tgHD model in the Libechev minipig. The model is characterized by high genetic homology to humans and a similar body mass and compartments. The minipig brain provides anatomical features that are attractive for imaging studies and could be used as endpoints for disease modifying preclinical studies similar to human HD. **RESULTS:** We demonstrate that complex MRI protocols can be successfully acquired with tgHD and wild type (wt) Libechev minipigs. We show that acquisition of anatomical images applicable for volumetric assessments is feasible and outline the development of a segmented MRI brain atlas. Similarly diffusion-weighted imaging (DWI) including fiber tractography is presented. We also demonstrate the feasibility to conduct in vivo metabolic assessments using MR spectroscopy. **CONCLUSIONS:** The results show that the minipig brain is well suited for MRI assessments in preclinical studies. We conclude that further characterization of phenotypical differences between tg and wt animals in sufficiently powered cross-sectional and longitudinal studies is warranted. **ACKNOWLEDGEMENT:** Funded by CHDI Foundation.

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GROUP B: HD MODELS

Neurochemical and neurophysiological correlate with motivational deficits in the rat BACHD Tg5 and mouse Q175 models of Huntington's disease**Joseph F. Cheer**

University of Maryland School of Medicine

Early symptoms of Huntington's disease (HD) include psychiatric impairments that precede motor deficits such as chorea and akinesia. Suppressed motivation represents one of the earliest and most common psychiatric symptoms in HD, but the neurobiology of this deficit is not known. Post-mortem and imaging studies in humans and measures of neuronal activity and dopamine release in animal HD models indicate that disrupted striatal dopaminergic function may support early, psychiatric symptoms, including motivational deficits. However, measures of compromised striatal neural activity and dopamine release in association with specific HD symptoms are lacking. Therefore, using the rat BACHD Tg5 and mouse Q175 models of HD, we sought to characterize mesostriatal function with in vivo freely-moving neurochemical and electrophysiological recordings in animals performing a motivational (progressive ratio, PR) task. Separate groups of animals were chronically implanted with carbon fiber electrodes in the nucleus accumbens (NAc) to measure subsecond dopamine release with fast-scan cyclic voltammetry or multielectrode electrophysiological arrays in the prefrontal cortex (PFC) and/or NAc to record extracellular local field potentials and single-unit cell firing. Both rat and mouse models of HD displayed significantly lower breakpoints under the PR schedule relative to WT controls. Impaired motivation was associated with a decrease in peak dopamine signal evoked by reward receipt. Genotypic differences in dopamine release manifested as trials progressed and cost increased and were greatest during the final trials of the PR session as animals reached their breakpoint. Motivational deficits also accompanied lower NAc single-unit encoding of reward delivery and disruptions in reward-evoked NAc gamma oscillations in HD versus WT animals. Further evidence of striatal dysfunction in HD animals include alterations in session-wide accumbal power spectra as well as cortico-striatal coherence. Overall, these findings suggest that profound deficits in striatal dopaminergic function and corticostriatal activity underlie the early motivational deficits inherent to HD and may serve as therapeutic targets in its treatment.

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GROUP B: HD MODELS

Standardized generation of patient-specific and gene-corrected iPSC lines for disease modeling and drug screening in neurodegenerative research**Lise Munsie**

Centre for Commercialization of Regenerative Medicine

The use of pluripotent stem cell-derived central nervous system and neuronal cell types for disease modeling, drug screening and regenerative medicine is an exciting area of activity in neurodegenerative research. Prior to the availability of pluripotent stem cells and associated methods for generating central nervous system and neuronal cell subtypes in vitro, relevant and affected primary cells have only been accessible post-mortem. Induced pluripotent stem cell (iPSC) technology enables the creation of patient-specific pluripotent cell lines, making personalized medicine approaches a possibility for people working on these disorders. Furthermore, recent advances in genome editing technologies promise the efficient creation of genetically corrected iPSC lines. At CCRM, we have established an iPSC production facility focused on generating high quality pluripotent cell lines from patient samples for academic researchers and clinicians. Fully operational for three years, CCRM has delivered over 85 patient iPSC lines that are being used for disease modelling, and in drug screening initiatives, at Institutes across Canada. Specializing in non-integrative reprogramming technologies, we have developed SOPs to reprogram many common cell types in feeder-free conditions, including dermal fibroblasts, bone marrow stromal cells, cord and peripheral blood, and endothelial cells. We are additionally developing the capabilities to perform genome editing in iPSCs using the newest and most efficient technologies, including TALENs and CRISPR/Cas9. These technologies can be used to induce a mutation of interest in control pluripotent cells or correct a genetic mutation in a patient-derived iPSC line. Access to patient-derived iPSCs and the associated isogenic line as a control would allow for the discovery of novel disease-associated phenotypes and therapeutic targets for specific patient subpopulations.

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GROUP B: HD MODELS

Characterization of new hTERT-immortalized Huntington's disease (HD) patient fibroblast cell lines**Claudia Hung**

McMaster University

HD is an autosomal dominant neurodegenerative disorder characterized by a triad of motor, cognitive and psychiatric symptoms. The disease is caused by a CAG expansion of >37 repeats in the huntingtin gene, manifesting as a polyglutamine expansion in the huntingtin protein. The study of HD cell biology in relevant neuronal cell types has been primarily limited to transformed SV40 large T-antigen-immortalized mouse striatal cell lines (STHdh) or primary neurons from mouse brains, each with their own restrictions and limitations. Culturing human brain cells from HD patients during disease progression is not feasible; instead, patient-specific fibroblast cells can be studied by extracting a skin biopsy. Primary fibroblasts are useful for studying cell biology in a clinically relevant context, and can also be converted to neurons by way of induced pluripotent stem cells (iPSCs). However, these fibroblasts have a limited number of passages before senescence. We sought to overcome this by immortalization with human telomerase reverse transcriptase (hTERT), which prevents degradation of chromosome ends during rounds of replication. We have immortalized fibroblasts from an HD patient (CAG repeats 43/17) and a spousal control wild-type patient (CAG repeats 21/18), to generate cell lines, termed TruHD cells. We are currently comparing TruHD cell phenotypes to known phenotypes of current models of disease and actual disease, including huntingtin localization, cell growth, phosphorylation levels and response to various stressors. Preliminary results show that phenotypes in TruHD cells are similar to those in primary fibroblast cells, validating this cell line as a viable research tool. Besides resolving problems with cell senescence, TruHD cell lines will be useful for the study of HD cell biology due to their improved immortalization protocol. The mechanism of immortalization for the STHdh cells involves altered p53 function, which can have downstream effects on huntingtin. hTERT-immortalization does not involve p53, making them ideal for studying the role of huntingtin in DNA damage and cell death pathways. Overall, these cell lines will be useful tools for the research community, with the potential to replace the most commonly used cell lines with a more clinically relevant model. Consistency between trials and ease of cell culture will be improved by using cell lines instead of primary cells. Additionally, efforts for direct conversion to neurons using small molecules have begun to generate patient-specific neurons.

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GROUP B: HD MODELS

Advancements for the study of striosome and matrix compartments in HD**Jill Crittenden**

Massachusetts Institute of Technology

Neuropathology in specific striatal circuits has been linked to HD symptoms. For example, early loss of striatal projection neurons in the indirect pathway (iSPNs) is observed in HD and is proposed to lead to hyperkinetic symptoms, as supported by animal models. Progressive loss of SPNs in the direct pathway (dSPNs) is thought to lead to increasing expression of akinetic symptoms. In addition to motor symptoms, individuals with HD show a range of emotional and cognitive problems. Loss of SPN dopamine receptors in HD, and dopamine imbalances, could contribute to these symptoms. SPNs classified according to their output pathways feature not only dSPNs and iSPNs, which make up most of the matrix compartment, but also a third SPN cell type (sSPNs) located in the striosome compartment, which project to the dopamine-containing neurons of the substantia nigra. Post-mortem evaluation of HD brains has shown that individuals with preferential degeneration of striosomes were more likely to have experienced predominant mood symptoms, relative to the motor symptoms seen especially in individuals with degeneration of the matrix compartment. We are developing tools to test the neurobiologic substrate for these potential differences in vulnerability in animal models, including mice engineered to render visible the striosome and matrix compartments of Q175 mice. We and others find that striatal matrix-enriched CalDAG-GEFI is down-regulated in HD and in mouse models of HD. Knock-down of CalDAG-GEFI was highly protective in a rat striatal-slice model of neuropathology induced by HTT expanded exon1, and over-expression of CalDAG-GEFI appeared to worsen pathology. CalDAG-GEFI knockout mice are prone to repetitive behaviors, but do not exhibit striatal loss. We found that scattered striatal SPNs that maintained high levels of CalDAG-GEFI in the R6/2 mouse model had smaller HTT nuclear inclusions than did SPNs with severe CalDAG-GEFI down-regulation. We have now crossed Q175 mice to CalDAG-GEFI-EGFP BAC mice to study, in striatal matrix neurons, potential links between varying levels of CalDAG-GEFI expression and varying levels of HTT inclusion formation.

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GROUP B: HD MODELS

Dual-gene CRISPR/Cas9 targeting to generate knockouts of potential Huntington's disease modifier genes in the mouse**Jacob Loupe**

Massachusetts General Hospital

Huntington's Disease (HD) is a dominantly inherited neurodegenerative disease, featuring motor signs, psychiatric disturbances and intellectual decline. The HD mutation is an unstable CAG trinucleotide repeat whose length is negatively correlated with the age at onset (AO) of diagnostic motor signs, accounting for ~65% of the substantial variance in AO. A genome wide association study (GWAS) by the GeM Consortium (Cell 162; 2015) carried out with four thousand HD individuals has now identified two chromosomal regions that each harbor an HD modifier gene: one on chromosome 15 and one on chromosome 8. Each region contains two genes, only one of which is likely to be the true modifier: MTMR10 or FAN1 (Chr 15) and RRM2B or UBR5 (Chr 8). To enable functional prioritization of these alternative modifier candidate genes *in vivo*, we have used a dual-gene CRISPR/Cas9 blastocyst injection strategy to rapidly generate independent lines of HD candidate modifier gene mutant mice. This allelic mutation strategy generated a spectrum of mutant alleles for each gene, some of which have (through germline transmission) yielded independent lines of Fan1, Mtmr10, Rrm2b and Ubr5 mice with indels (in frame or truncating) mutations. The pros and cons of this dual-targeting approach will be presented. The lines of mice that we have created will provide an important resource for 1) genetic crosses with Hdh CAG knock-in mice to functionally prioritize each candidate as a modifier of CAG repeat instability and disease phenotypes that become evident in these precise genetic models of the HD expansion mutation and 2) for detailed studies of the function of the true HD modifier genes whether they are discovered in the mouse strategy or through ongoing studies in HD patients.

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GROUP B: HD MODELS

Novel multi-modal endpoints powered for pre-clinical studies in the B6.HttQ111/+ model of Huntington's disease**Jeffrey B. Carroll**

Western Washington University

We are developing endpoints powered to detect therapeutic efficacy in trials with knock-in mouse models of HD. Towards this aim, we characterized histological and transcriptional profiles of cohorts of B6.HttQ111/+ (hereafter HttQ111/+) mice at 3-, 9-, and 12-months of age. Striatal neuropathology revealed a progressive accumulation of large mutant HTT (mHTT) neuronal intranuclear inclusions (NIIs) from 0% at 3-months to 12% at 9-months and finally 28% at 12-months. In addition, the average size of aggregates increased 40% between 9- and 12-months of age. Immunoreactivity of medium spiny neuronal marker DARPP32 is reduced by 45% per cell in HttQ111/+ compared to Htt+/+ at 9- months and further reduced by 71% at 12-months. Striatal cell counts reveal no reduction in the numbers of neuron per area through 12-months of age, however the ratio of neurons:total cells per square micrometer in the dorsolateral striatum is reduced 5.7% at 9- months and remains reduced at 12-months. We also conducted an unbiased analysis of striatal and cerebellar transcriptomes at 3- and 9- months of age using RNA sequencing. Very few transcriptional changes are observed over this time course in cerebellar tissue, while 9-month striatal tissue revealed a number of significant transcriptional alterations in HttQ111/+ mice. Genes involved in a number of biological pathways of interest, notably neurotransmission, were down-regulated in 9-month old HttQ111/+ striata. We validated a number of these changes using QRT-PCR. DARPP32 expression was normal at 3- months of age, but reduced at both 9- and 12-months ($p = .01$). Additional neuronal signalling and synaptic markers are also normal at 3-months and reduced at 9- and 12- months. Other genes, including the microglia-enriched gene H60b, the immunoglobulin response gene Islr2, and the DNA damage involved gene N4bp2, are normal at 3-months but increased in 9- and 12- month HttQ111/+ mice. To assess the usefulness of these phenotypes as endpoints for preclinical studies with HttQ111/+ mice, we conducted simulation studies assuming a range of levels of rescue for each endpoint. These analyses reveal even modest rescue (10-25%) for many of the HD-specific molecular changes can successfully be detected in a preclinical study with a small N per arm (N=10/arm in a genotype x treatment design). Taken together, we believe that we have established the HttQ111/+ mouse as a useful tool in testing the preclinical efficacy of novel HD therapeutics targeting the earliest molecular consequences of mutant Htt expression in the striatum.

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GROUP B: HD MODELS

Novel screen to identify therapeutic compounds for Huntington's disease**Alessia Deglincerti**

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We have developed a technology (micropatterns) that allows high-resolution, dynamic analysis of cellular behaviors. When grown on micropatterns, human embryonic stem cells (hESCs) self-organize into different tissue types that generate a specific signature defined by geometry and size. This signature is driven by processes that occur during human development and recapitulates cell-cell signaling and the relationship between cells as they differentiate to give rise to all cell types. Disease-causing mutations change this signature. Using CRISPR/Cas9 genome-editing technology, we have generated Huntington's Disease-(HD) hESC lines that are genetically identical to their normal counterpart except for the polyQ expansion ("isogenic lines") and we have identified an HD-specific micropattern signature that is significantly different from its normal isogenic counterpart. We are now using this platform to screen for potentially therapeutic compounds that revert the HD signature back to normal.

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GROUP B: HD MODELS

HD modelling by using PSCs 2D and 3D differentiation into striatal and cortical domains**Elena Cattaneo**

University of Milano

In order to better understand the mechanisms that underlies HD, we have developed a strategy by harnessing the power of pluripotent stem cells (PSCs) differentiation into multiple classes of neurons and to self-assembly into complex structures. First, we have been studying the differentiation of PSCs into MSNs by exploiting two secreted proteins, Shh and Dkk1, which are responsible in vivo for the correct patterning of ventral telencephalic structures. By incorporating these molecules into the dual-SMAD inhibition protocol we were able to differentiate multiple PSCs lines into MSNs as described in our paper (Delli Carri et al., 2012) and in many new differentiation experiments (n=30) performed by five scientists during a three-year program (unpublished). The progression of PSCs towards MSNs has been dissected by analyzing key telencephalic markers at multiple time points in multiple cell lines. We developed cell quantification procedures to give a precise assessment about the number of positive cells for each marker at each time point. PSCs differentiated with this MSNs-specific protocol were transplanted in a rat model of HD where they survived, matured, and integrated into the host system (Besusso, Faedo, in preparation). Next, we applied this well-characterized protocol to control and HD-derived iPSC cell lines, with the aim of studying how striatal differentiation changes according to the CAG length (Conforti, in preparation). In parallel to this morphogens-based differentiation protocol, we developed hES-inducible lines (TETON-based) that can express key striatal transcription factors (TFs) in defined temporal windows. In particular, by co-expressing both Gsx2 and Ebf1 during a critical window of neuronal specification, we provided the proof-of-principle that these two TFs can be harnessed to increase efficiency of MSNs production (Faedo, in preparation). A modified-mRNA strategy is currently under development to extend these findings to other PSCs lines. Secondly, we decided to start addressing the cerebral cortex component in HD by subjecting iPSC cells to a cortical-specific protocol (Shi et al., 2012), with a systematic procedure similar to the striatal protocol described above. Preliminary results point to defects similar to those seen in the MSNs differentiation protocol, thus reinforcing the idea of a neural developmental defect in HD. Thirdly, we took advantage of the self-assembly potential of PSCs to start modeling HD in a more complex 3D-based differentiation protocol. Our strategy consists of exploiting the published cortical organoid protocol (Lancaster et al., 2013) in order to extend our cerebral cortex dysfunction 2D-analysis.

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GROUP B: HD MODELS

Tissue specific HTT aggregation load is associated with impairments of mitochondrial biology in the R6/2 mouse model**Tanja Hering**
Ulm University

The HTT CAG repeat expansion mutation that causes Huntington's disease (HD) is present in every tissue, even though HD seems to primarily affect the brain. It is therefore possible that the impact of the mutation on HTT protein biology, but also on biological systems, is tissue specific. It has been reported that in skeletal muscle tissue of the R6/2 fragment mouse model mutant HTT (mHTT) formed aggregates, while in cardiac muscle tissue no aggregates could be detected (1). Therefore, the aim of our study was to test the hypothesis that the differences in aggregate formation might drive the differences in dysregulation in a biological system. To this end we characterized as an example the mitochondrial biology (RNAseq, protein levels, function) of skeletal muscle compared to cardiac muscle tissue in the R6/2 mouse model. We found that in skeletal muscle (quadriceps femoris, tibialis anterior) of late-stage R6/2 mice the majority of genes relevant for oxidative phosphorylation, mitochondrial biogenesis or mitochondrial dynamics were simultaneously down regulated at the transcript level. At the protein level, parts of the TCA cycle, mitochondrial biogenesis and the fission/fusion balance were dysregulated. Complex IV activity was deficient and mitochondrial fission/fusion balance shifted towards increased fission resulting in a larger number of mitochondria. In contrast to skeletal muscle the mitochondrial transcriptome, protein levels and OXPHOS function were largely normal in heart muscle of end-stage R6/2 mice. Our data suggest that the impact of mutant huntingtin on mitochondrial biology differs between skeletal and heart muscle tissue with the extent of huntingtin aggregate formation potentially playing an important role. 1) Mielcarek M, Inuabasi L, Bondulich MK, Muller T, Osborne GF, Franklin SA, et al. (2014) Dysfunction of the CNS-Heart Axis in Mouse Models of Huntington's Disease. *PLoS Genet* 10(8): e1004550. doi:10.1371/journal.pgen.1004550

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GROUP B: HD MODELS

The kynurenine 3-monooxygenase inhibitor Ro 61-8048 does not protect against 3-nitropropionic acid induced neurotoxicity in rats**Michel Alexander Steiner**
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Kynurenine (KYN), a major metabolite of tryptophan, is converted into kynurenic acid (KYNA) and 3-Hydroxykynurenine (3-HK) through the enzymes kynurenine aminotransferase (KAT) and kynurenine 3-monooxygenase (KMO), respectively. Whereas KYNA in the brain has neuroprotective effects, 3-HK is further metabolized into quinolinic acid (QUIN) which is neurotoxic at high concentrations. Blocking KMO by small molecule inhibitors can lead to indirect neuroprotection by inhibiting the formation of QUIN and at the same time increasing the concentration of KYNA. JM6, the proposed pro-drug of the KMO inhibitor Ro 61-8048, slows down neurodegeneration and increases survival and motor function in the R6/2 mouse model of Huntington's disease [HD] (*Cell* 145:863-874, 2011). KMO inhibition could also be a relevant treatment strategy in human HD, where QUIN is up-regulated in striatum, and KYNA down-regulated in the cerebrospinal fluid (CSF). 3-nitropropionic acid (3-NP) is a non-competitive inhibitor of mitochondrial succinate dehydrogenase (complex II), which leads to selective neurodegeneration in the striatum in both rodents and humans. It causes similar behavioral motor symptoms in rats as those that occur in HD patients. Exposure to 3-NP mimics the mitochondrial dysfunction associated with expression of mutant Huntingtin and was proposed as a pharmacological model of HD. The aim of the current study was to evaluate whether KMO inhibition through Ro 61-8048 or its pro-drug JM6 could attenuate disease symptoms in the 3-NP rat model of HD. Preparatory pharmacokinetic experiments showed that after oral gavage in rats JM6 is actually not converted into Ro 61-8048, and does not act as a KMO inhibitor. Ro 61-8048, on the other hand, was orally available and increased KYNA and its precursor KYN in periphery and CSF suggesting potent KMO inhibition in vivo. When Lewis rats were exposed to 3-NP (45 mg/kg/d delivered via osmotic minipumps) in combination with 10 or 50 mg/kg (p.o., once daily) of Ro 61-8048 as compared to vehicle during 2 weeks, the 3-NP-induced motor deficits, body weight loss, and percentage of rats becoming moribund was not reduced despite a 6-fold increase of KYNA in CSF. We conclude that, first, JM6 is not a pro-drug of Ro 61-8048 and not itself a KMO inhibitor in rat. This corroborates findings of CHDI in mice (*DMD* 40:2297-2306, 2012). Second, active doses of the KMO inhibitor Ro 61-8048 are not neuroprotective in the 3-NP rat model of HD. The extent to which 3-NP induced neurotoxicity involves the kynurenine pathway remains to be investigated.

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GROUP B: HD MODELS

A clinical phenotype-based time-to-event model to include undiagnosed HD individuals in genetic discovery of age at onset modifiers**James Gusella**

Massachusetts General Hospital

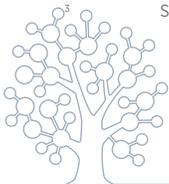
Huntington's disease (HD) is caused by a CAG repeat expansion mutation whose size is the primary determinant of the rate of the pathogenic process that leads to disease onset. Recently, the GeM-HD Consortium showed that some of the remaining variation in age at onset is due to genetic variation in other regions of the genome that is capable of hastening or delaying diagnostic disease manifestations. These modifiers, which act prior to emergence of clinical disease, promise to provide new, in-human-validated targets for development of therapies to prevent or delay HD onset. To date, the quantitative genome-wide association (GWA) approach to identifying onset modifiers has relied exclusively on diagnosed HD subjects, comparing their observed onset age with that expected based upon the size of their CAG expansion mutation. Since the power of GWA studies increases dramatically with increasing sample size, we sought a route to also include HD expansion carriers who have not yet been clinically diagnosed, such as the individuals in the PREDICT-HD cohort. Recently, development in PREDICT-HD of a prognostic index for predicting high diagnostic confidence level (DCL=4) showed that clinical measures and CAP (CAG x Age Product) score have distinct predictive ability. Consequently, we constructed a time-to-event model for estimating the timing of future onset using total motor score (TMS) and Symbol Digit Modalities Test (SDMT) data, but explicitly omitting CAG length and age. For those individuals predicted to have disease onset within 10 years, the model provides a maximum likelihood estimate that, when added to their current age, yields an estimated age at onset that can be used, in conjunction with CAG-length predicted onset, for modifier studies. Inclusion of these as yet undiagnosed individuals in GWA onset modifier analysis increased the significance of confirmed modifier SNPs, demonstrating that the predictive survival model method captures valid modifier information. This approach can therefore be used to increase the sample size and the power of modifier GWA analysis. In addition, comparison of the predicted age at onset with CAP score provides an effective approach to delineating age at onset outliers for additional genetic studies, such as exome sequencing or dichotomous association analysis.

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GROUP B: HD MODELS

Elevated urea in HD patients and the OVT73 sheep model – a novel biological process for therapeutic intervention**Suzanne Reid**

The University of Auckland

Whilst the causative mutation in HD is well understood, the means by which this confers pathology is less defined. We make use of the OVT73 sheep model to better understand the early pathogenesis of HD, as it is a long lived large animal with modest mutant huntingtin expression levels carrying a moderate repeat of 73 units [1, 2]. They are outwardly healthy and indistinguishable from control siblings, but demonstrate early brain pathology including aggregates [2], and a circadian disruption [3]. Recently, an RNAseq experiment and subsequent nCounter RNA quantification identified elevated SLC14A1 transcripts in transgenic sheep striatum compared to controls (2.2 fold increase, p-value =0.007). SLC14A1 codes for a urea transporter (UTB), and using an enzymatic assay we find elevated urea levels within the transgenic sheep striatum. Urea is formed by arginase-mediated cleavage of arginine within the final step of the urea cycle, which is critical in dealing with amine-nitrogen such as ammonia, which is highly neurotoxic. Urea is predominantly produced in the liver via UTA, and is usually rapidly excreted by the kidneys into the urine. However, UTB expression is widespread, particularly within astrocytes, indicating the importance of urea transport within the brain; indeed many neurological conditions are the result of accumulation of ammonia. Intriguingly, urea has recently been reported as severely elevated (3-4 fold) throughout human patient brain tissue [4], and our preliminary data show that levels of the UTB protein are also elevated in human disease brain. Here we present evidence in support of use of the OVT73 sheep model as a prodromal testing system, and hypothesize that strategies to reduce ammonia levels may ameliorate HD pathogenesis. 1. Jacobsen, J.C., et al., An ovine transgenic Huntington's disease model. *Hum Mol Genet*, 2010. 19(10): p. 1873-82. 2. Reid, S.J., et al., Further Molecular Characterisation of the OVT73 Transgenic Sheep Model of Huntington's Disease Identifies Cortical Aggregates. *J Huntingtons Dis*, 2013. 2(3): p. 279-295. 3. Morton, A.J., et al., Early and progressive circadian abnormalities in Huntington's disease sheep are unmasked by social environment. *Hum Mol Genet*, 2014. 23(13): p. 3375-83. 4. Patassini, S., et al., Identification of elevated urea as a severe, ubiquitous metabolic defect in the brain of patients with Huntington's disease. *Biochemical and Biophysical Research Communications*, 2015.

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[GROUP B]

Poster Abstracts

Systems Biology

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GROUP B: SYSTEMS BIOLOGY

Spatiotemporal proteomic profiling of mutant huntingtin inclusion bodies in vivo reveals widespread protein loss-of-function**Fabian Hosp**

Max Planck Institute of Biochemistry

Aggregation of misfolded gene products is a central hallmark of protein conformational disorders. Huntington's disease (HD) is characterized by the aggregation of polyglutamine-expanded huntingtin (HTT), proceeding from soluble oligomers to end-stage inclusions. How protein aggregation leads to neuronal dysfunction is not well understood. To address this question, we employed mass spectrometry-based proteomics with spatiotemporal resolution to identify neurodegeneration signatures at the systems level in the R6/2 mouse model of HD. First, we found that extensive remodeling of the soluble brain and cerebrospinal fluid proteome correlated with changes in HTT aggregate formation in a time- and brain region-dependent manner. Second, quantitative proteomic profiling of mutant HTT inclusion bodies uncovered an unprecedented complexity of several hundred proteins that are consistently sequestered during disease progression. Third, we observed a significant enrichment of extended low-complexity sequence regions and other aggregation-prone sequence motifs in these inclusion-associated proteins, highlighting their intrinsic propensity to facilitate co-aggregation with other proteins. Hence, our complementary proteomic approach enabled us to identify hundreds of potential loss-of-function candidates. Fourth, experimental rescue of several candidates ameliorated polyQ-mediated toxicity in a neuron-like cell line and, surprisingly, showed an effect on both the size and the morphology of HTT inclusions. In summary, our study provides a highly comprehensive spatial and temporal view of dynamic proteome changes during HD pathogenesis, indicating that widespread loss of protein function contributes greatly to aggregate-mediated toxicity.

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GROUP B: SYSTEMS BIOLOGY

HINT: A novel integrative platform for Huntingtin Interactions**Jefferson Parker**

Thomson Reuters

The use of systematic protein-protein interaction detection methods over the past decade has facilitated a wide range of putative functions to be associated with the huntingtin (HTT) protein. This list includes, but is not limited to, trafficking & transport, actin dynamics, autophagy and chaperoning. However no consensus has been reached on the precise biological activity modulated by HTT. To provide a more clear-view into the molecular mechanisms that are consistently related to HTT, we curated and analyzed data from over 100 published studies containing huntingtin protein interactions detected using various methods (antibody pull-down, yeast two hybrid, etc.) as well as from leading protein-protein interaction databases (BIOGRID, IntAct, HPRD etc.). Here we present a first-of-its-kind, searchable database of HTT interactions (HINT) as a step towards further advancing our understanding of huntingtin network biology. Through this database we provide an integrated and standardized view of all HTT interactions available across a variety of molecular interaction databases and literature articles published between 1995 and 2015. By relying on industry standard ontologies such as Entrez, HUGO, Gene Ontology (GO) and the Proteomics Standards Initiative (PSI-MI), this database semantically integrates cross-platform interaction information. Furthermore, through its integration with GO and pathway databases such as Kegg, Reactome, Wikipathways, Biocarta, Humancyc, PharmGKB, SMPdb and Netpath, this database can also serve as a novel, enabling resource for the HD research community to perform gene enrichment analyses, ontological classification and visualization using HTT interactors. As the first comprehensive compendium of Huntingtin protein interactors, HINT can guide experimental and computational scientists to biology most proximal to HTT. Additionally, HINT can assist in the identification of tractable drug targets within the Huntingtin interaction network, as well as support hypothesis generation with regard to new pharmacodynamic and clinical biomarkers for a drug target of interest. Finally, HINT will be made available to the community on CHDI's HDinHD web platform.

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GROUP B: SYSTEMS BIOLOGY

Long-term improvement in motor deficits and striatal neural activity in heterozygous Q175 mice following up-regulation of striatal EAAT2 (GLT1) by AAV9**George V. Rebec**
Indiana University

Dysfunctional glutamate transmission has been implicated in multiple neurodegenerative conditions, including Huntington's disease (HD). EAAT2 (GLT1), the protein found primarily in astrocytes and responsible for clearing >90% of glutamate from brain extracellular fluid, is down-regulated in both HD patients and transgenic mouse models of HD. Reversing the loss of EAAT2 by treatment with ceftriaxone, a beta-lactam antibiotic, temporarily improves the mouse HD phenotype (B.R. Miller et al., 2008, *Neuroscience*, 153:329-337). Here, we used intravascular injections of AAV9-EAAT2 to induce a long-term increase in brain EAAT2 expression. Within 6 weeks after injection in adult (25 weeks of age) Q175 mice, EAAT2 expression increased in both heterozygous (HET) and homozygous (HOM) mice, reversing the EAAT2 decline normally associated with this model. Immunohistochemistry confirmed an AAV9-mediated increase in EAAT2 expression in striatal astrocytes. Multiple behavioral tests (rotarod, open-field movement, plus-maze turning, and nest building) were begun at 30-32 weeks of age and now indicate a marked improvement, including a delay in symptom onset, as HETs approach 50 weeks of age. HOMs also show improvement, but not as consistently as HET mice. Ongoing recording of spike and local field potential (LFP) activity in the striatum of the same animals revealed corresponding neural changes relative to untreated Q175s. In both HET and HOM mice, for example, patterns of spike bursting and behavior-related changes in LFP signaling began to resemble the striatal electrophysiology of healthy wild-type controls. Collectively, these data confirm and extend research suggesting a role for glutamate dysfunction in the neurobehavioral pathology of HD and focus attention specifically on deficits in EAAT2 expression as a potential target for HD therapeutics.

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GROUP B: SYSTEMS BIOLOGY

Gene expression dysregulation in Huntington's disease: potential involvement of nuclear lncRNAs**Pamela Milani**
Massachusetts Institute of Technology

Increasing evidence has shown that gene expression dysregulation is an important feature of Huntington's disease (HD). Here we combine next-generation sequencing (NGS) techniques with biochemical approaches and functional experiments to gain further mechanistic insights into the molecular relationship between chromatin modifications and altered gene expression programs in HD. First, we used the assay for transposase-accessible chromatin using sequencing (ATAC-Seq) to interrogate chromatin accessibility genome-wide in cortex brain tissue from male R6/1 mice and sex-matched wild-type littermates. We detected widespread changes in the epigenetic signals: 4,687 genomic sites were differentially enriched between mutant and wild-type animals (p -value threshold=1E-05). Second, we performed total RNA-sequencing experiments using ribosomal RNA depletion and a strand-specific library preparation protocol that allowed us to retain information about the directionality of each transcript. We detected 2,050 differentially expressed genes (DEGs - q -value threshold=0.05), the majority of which (1284) were down-regulated and enriched in GO terms such as regulation of ion transport (FDR=4.65E-19), synaptic plasticity (FDR=1.30E-07) and neuron differentiation (FDR=2.09E-04). Interestingly, among the DEGs, we identified 95 lncRNAs, which include pseudogenes, antisense transcripts, and nuclear-retained lncRNAs (nr-lncRNAs). Notably, recent observations have highlighted the roles of nr-lncRNAs in modulating gene expression events through the interaction with chromatin-modifying enzymes, transcription factors, and RNA-binding proteins; in addition, several neuronal-enriched nr-lncRNAs have been shown to play important roles in neural differentiation, neurite outgrowth and synaptogenesis. Therefore, it seems plausible that altered levels of these lncRNAs can modify the topological and functional organization of nuclear structures and affect gene expression processes with important implications for HD. To test this hypothesis we are currently modulating the expression of some of these dysregulated nr-lncRNAs in primary mouse cortical neurons and carrying out quantitative assessment of neurite outgrowth and synaptogenesis using high-content imaging. Furthermore, we are performing capture hybridization analysis of RNA targets (CHART) coupled with NGS in cortex brain tissue from mice to isolate and map the DNA sites associated with our candidate lncRNAs. The goal is to test whether lncRNA genomic binding sites overlap with regulatory elements at which changes of chromatin structures and accessibility have occurred and are associated with, and therefore potentially causative of, transcriptional program variations in HD.

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GROUP B: SYSTEMS BIOLOGY

Striosomes may control dopamine activity via disynaptic projections to the lateral habenula**Simon Hong**

Massachusetts Institute of Technology

Huntington's disease can induce debilitating disorders of mood and affect that can precede motor impairments for which HD was originally noted. Here we sought to define a neural circuit that could underlie such symptoms. The lateral habenula (LHb) influences motivation and behavior via its influence on the dopamine (DA) and serotonin (5HT) systems (e.g., Hong et al., 2011). In non-human primates, the LHb receives a major input from the border neurons in the globus pallidus internal segment (GPb; Hong et al., 2008). A leading hypothesis is that the GPb cells might receive input from neurons in the striosomal compartment of the striatum (Graybiel and Ragsdale, 1978), given evidence in rodents (Rajakumar et al. 1993). Definitive evidence of such a circuit in the primate brain is still missing. To fill this major gap, in two rhesus monkeys, we placed microelectrodes in the striatum for microstimulation and placed a second microelectrode in the LHb for recording the responses to striatal microstimulation. We mapped different microstimulation sites in the striatum (to which we applied 3 pulses, 33 μ A each, at 300 Hz), and determined whether the microstimulation affected multi-unit activity in the LHb, indicating functional connectivity. We found that at some sites stimulation of the striatum induced significant inhibitions or excitations of LHb activity. These effective striatal sites were distributed unevenly along the depth of the stimulating electrode's track; sometimes a brisk excitement or inhibition in the LHb was induced, but at other depths no LHb response occurred. We made electrolytic marking lesions when we found an effective site, and the brains of the two monkeys were then processed for histological examination using KChIP1 (to demonstrate striosomes), glial fibrillary acidic protein (GFAP, to allow detection of the penetration track) and Prussian Blue (to detect iron deposition at marking lesions). Our analyses demonstrated that 9 out of the total of 11 effective sites were in or bordering striosomes, one was in the matrix compartment, and one was in a fiber bundle. The rather long orthodromic latency of ~20ms for LHb responses suggested disynaptic connectivity, contrasting with the ~5 msec latency for GPb cell stimulation. Our results strongly suggest that the striosomal compartment of the striatum controls LHb activity via its connection to the GPb. This work, still in progress, has the potential to identify in non-human primates a crucial pathway for mood control, one likely targeted in HD patients. Funded by CHDI.

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GROUP B: SYSTEMS BIOLOGY

Genome-wide mapping of the binding sites of striatal-enriched transcription factor Kruppel-like factor 16 from in vivo mouse striatal tissue**Jocelynn Pearl**

Institute for Systems Biology

Transcriptional changes have long been identified as some of the earliest detectable signatures in Huntington's disease (HD). Multiple datasets spanning human post-mortem tissue as well as mouse models of HD have demonstrated dramatic shifts in brain gene expression. Changes in coordinated sets of differentially expressed genes can be assessed using gene set enrichment analysis or through identification of gene co-expression networks. While these approaches yield pathway or biological process hypotheses about HD, they do not generally provide immediate insights into specific potential regulators for follow up experiments. By contrast, transcriptional regulatory networks (TRNs) reduce complexity and provide a biological framework for follow-up experimental investigation of candidate transcription factor drivers in HD. TRN models make mechanistically and quantitatively informed predictions about the targets of each transcription factor (TF) in the mouse and human brain by integrating transcriptomics data with evidence from cis-motifs and genome-wide DNase hypersensitivity footprinting. Our TRN model identified Kruppel-like factor 16 (Klf16) as a key driver of transcriptional change in the striatum of both HD mouse models and human HD caudate. Klf16 (also known as the dopamine receptor regulating factor) is a zinc-finger transcription factor that is enriched in brain regions receiving dopaminergic input. We mapped the genome-wide binding locations of Klf16 in both wildtype and CAG-expanded Htt mice from in vivo mouse striatal tissue using high resolution X-ChIP-seq. X-ChIP-seq improves chromatin fragmentation using light MNase digestion. Chromatin immunoprecipitation is then followed by high-throughput sequencing to generate maps of genome-wide protein binding. In our initial ChIP-seq study, we found 1493 Klf16 binding sites across the genome. Our ongoing work to generate high quality binding maps for Klf16 will further clarify the biological pathways and direct targets of this important regulator. Defining early regulatory state differences in accurate genetic HD mouse models allow us to clarify the transcriptional changes we observed in longitudinal transcriptomic studies in these same mice. The data from these studies enable us to study 'pre-manifest' HD, and clarify molecular changes that occur well before the onset of overt symptoms with the potential of advancing new therapeutic targets or clinical endpoints.

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GROUP B: SYSTEMS BIOLOGY

Modeling the earliest consequences of the HD mutation in the mouse**Douglas Barker**

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The expanded HTT CAG repeat is the root genetic cause of Huntington's disease (HD). The inverse relationship of CAG repeat size with the age at onset provides a genetic-based strategy for identifying the earliest events in the disease process. Such events are expected to hew to the key HD genetic parameters: true dominance, progressivity with CAG size and striatal vulnerability. To identify the earliest gene expression changes that fit the HD criteria, we have performed analyses of genome-wide RNA-sequence datasets generated from cohorts of heterozygous B6.Htt CAG expansion knock-in allele mice, with a range of CAG allele sizes (named Q20, Q50, Q80, Q92, Q111, Q140, Q175) that have been deeply phenotyped at the behavioral and the molecular levels, at three ages (2, 6 and 10 months). Our main goal is to understand the earliest effects of CAG repeats that cause the majority of HD - 41-50 units, but the available current allelic series is skewed towards very long alleles, which are relatively rare in the HD population. Consequently, in contrast to the traditional strategy, where the longest CAGs are compared with the shortest alleles at ages when a large proportion of the transcriptome is altered, our phased approach aims to enrich for those genes whose expression is changed in a CAG-dependent fashion across the shortest alleles in this series, at the youngest age (2 months). Our strategy and analysis methods will be presented. The early/low CAG gene sets that we have identified afford clues to the biological processes that are perturbed by the mutation before the transcriptome is more broadly dysregulated and their evaluation within causal network models generated from the allelic series datasets provides a route to understanding and predicting the potential network-wide consequences of HD early-gene dysregulation.

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GROUP B: SYSTEMS BIOLOGY

Genome-scale transcriptional regulatory network models for the mouse and human striatum reveal transcription factors underlying Huntington's disease**Seth Ament**

Institute for Systems Biology

Transcriptional regulatory changes are thought to play an important role in Huntington's disease (HD) pathogenesis, yet the roles for specific transcription factors (TFs) remain poorly characterized. We reconstructed a genome-scale model for TF-target gene interactions in the mouse striatum by integrating a physical model of transcription factor binding sites in the mouse genome with large-scale transcriptome profiling of striatal tissue from HD mouse models. This model accurately predicted the physical binding sites of TFs based on ChIP-seq, and it made quantitatively accurate predictions for the expression of 13,009 genes in the striatum. We identified 46 down- or up-regulated TF-target gene modules that showed progressive, age- and Q-length-dependent enrichments for differentially expressed genes in the striatum of HD mouse models. Down-regulated TF-target gene modules were overrepresented for genes specifically expressed in medium spiny neurons and for genes involved in synaptic functions. Up-regulated TF-target gene modules were overrepresented for genes specifically expressed in oligodendrocytes or astrocytes and for genes involved in apoptosis and DNA repair. Network analyses of human striatum suggested that many of these TFs are also important regulators in late-stage human disease. These results suggest that HD pathogenesis involves changes in the activity of numerous TFs in the striatum, both in neurons and in glia. This approach for the reconstruction and analysis of TF-target gene interactions is likely to be broadly applicable for the characterization of TFs underlying mammalian phenotypes.

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GROUP B: SYSTEMS BIOLOGY

Multi-omic analysis of B6.HttQ111/+ hepatocytes reveals insensitivity to high-fat feeding**Jeffrey B. Carroll**

Western Washington University

We are interested in identifying the earliest molecular consequences of expression of endogenous levels of a single allele of mutant Huntingtin. We are using targeted environmental perturbations in an attempt to highlight vulnerabilities induced by mutant HTT exposure that may not be obvious under baseline conditions. As a model environmental perturbation, we have exposed wild-type and HttQ111/+ mice to a range of dietary fat (10%, 45% and 60% kcal/fat) from weaning to 90 days of age and examined the molecular consequences in primary hepatocytes. Hepatocytes normally dramatically remodel their transcriptome and cellular metabolism in response to increased dietary fat, providing a convenient model system to test the hypothesis that mutant HTT will render cells differentially sensitive to metabolic stress. Molecular profiling of hepatocytes included: transcriptome profiling with RNA sequencing, lipidomic profiling by LC-MS and targeted analysis of metabolic flux from ¹³C-glucose to specific product metabolites using GC-MS. Consistent with previous observations, a wide range of transcriptional changes are induced in hepatocytes by high-fat feeding (> 1,300 transcripts altered with FDR < 0.1). Surprisingly, 1,084 of these transcriptional changes were observed in wild-type but not HttQ111/+ cells - conversely, very few diet-induced transcriptional changes (20 transcripts) were observed in HttQ111/+ cells but not wild-type cells. We will present pathway enrichment analyses, including data supporting genotype-specific alterations in predicted transcription factor activation by dietary fat using a novel transcription factor regulatory network model. Lipidomic analysis also reveals a wide-range of changes induced by high-fat feeding (of 394 assayed lipids, 216 had a nominally-significant response to diet). While only a single lipid species assayed had a robust response to genotype, 54 species had a significant genotype by diet interaction, consistent with a genotype-specific response to high-fat feeding. In nearly every case, on individual investigation these lipids were found to be altered by dietary fat in wild type, but not HttQ111/+ cells. In wild-type hepatocytes, we find that high-fat diet leads to a robust reduction in complete ¹³C-glucose oxidation to ¹³CO₂, consistent with a down-regulation of glucose catabolism in favor of fatty acid oxidation. This diet-induced metabolic shift is completely absent in HttQ111/+ cells. In short, we observe that primary hepatocytes from 90 day old HttQ111/+ mice have a marked insensitivity to molecular changes normally induced by high-fat feeding, suggesting that mHTT may impair lipid metabolism or fuel selection, more generally, in these cells.

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GROUP B: SYSTEMS BIOLOGY

Data-driven reconstruction and simulation of transcriptional regulatory networks in the HTT allelic series**Michael Beste**

GNS Healthcare

Allelic expansion within HTT gives rise to a progressive striatal pathology involving transcriptional dysregulation, impaired protein folding and degradation, disrupted neurotransmission, and mitochondrial dysfunction. Outside of the striatum, the cascade of molecular effects are less well-characterized, but include both potentiating and compensatory effects to striatal degeneration. In collaboration with CHDI, we have constructed a comprehensive family of REFS (Reverse Engineering Forward Simulation) Bayesian network models linking RNAseq profiles of the striatum, cortex, hippocampus, and cerebellum to behavioral endpoints for the murine Htt allelic series to identify the earliest molecular effectors of CAG-repeat expansion. Inferred model ensembles structurally orient upstream drivers towards their downstream targets in a tissue-specific manner, and forward simulations enable genome-wide perturbation analysis to predict the principal regulators intersecting Htt sub-networks. In addition to early striatal regulators of cell survival (e.g. Pde1b and Adcy5), simulations identified a prominent cerebellar response network encompassing both striatal and novel transcriptional regulators involved in related trinucleotide repeat disorders, including Atn1, Atxn1, and Rest. We further describe simulation network validation results using orthogonal data sets, as well as efforts aim to expand existing RNAseq models with their proteomic complement and measurements of somatic instability at the CAG locus.

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[GROUP B]

Poster Abstracts

Translational Medicine

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GROUP B: TRANSLATIONAL MEDICINE

Preventing calcium dysregulation and synaptic loss in Huntington's disease: an evaluation of sigma-1 receptor agonists in corticostriatal co-cultures from YAC128 mice**Daniel Ryskamp**

UT Southwestern Medical Center

In Huntington's disease (HD), mutant Huntingtin (mHtt) causes early corticostriatal synaptic dysfunction and eventual neurodegeneration of striatal medium spiny neurons (MSNs). There are no disease-modifying treatments for HD, but ongoing clinical trials with Pridopidine show promise for improving motor symptoms. Also, Pridopidine is neuroprotective in R6/2 HD mice. Yet, the target of Pridopidine and its mechanism of action are unclear. As recent binding studies identified Sigma-1 receptor (S1R) as a high affinity receptor for Pridopidine, we evaluated the relevance of S1R as a therapeutic target. S1R is an endoplasmic reticulum (ER)-resident transmembrane protein and changes in its expression or genetic sequence are associated with neurodegenerative phenotypes. S1R activity is regulated by ER Ca²⁺ homeostasis, which is perturbed in HD, making it likely for S1R activity to also be abnormal. Consistent with this we observed compensatory upregulation of S1R protein in striatal samples from aged YAC128 HD mice and HD patients. We used corticostriatal co-cultures to examine mechanisms of age-dependent dendritic spine loss in MSNs from YAC128 mice. The commercially available S1R agonist 3-PPP, which has a very similar chemical structure to Pridopidine and an identical affinity to S1R, completely prevented MSN spine loss in YAC128 co-cultures (100 nM for 16 hours on DIV21). This rescue was blocked by knockout of neuronal S1R using lenti-viruses to express Cas9/gRNA targeting mouse S1R. Expression of human S1R restored protection by 3-PPP. We previously found that mHtt sensitizes the ER Ca²⁺ channel InsP3R1, resulting in depletion of ER Ca²⁺ and elevation of store-operated Ca²⁺ entry in MSNs to synaptotoxic levels. We now report that 3-PPP normalizes YAC128 MSN Ca²⁺ homeostasis by suppressing InsP3R1 hyperactivity. S1R knockout prevented replenishing of ER Ca²⁺ by 3-PPP. This reveals a potential mechanism of action for S1R agonists and highlights S1R as a target for HD.

Jun Wu, Lili Wu, Ilya Bezprozvanny

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GROUP B: TRANSLATIONAL MEDICINE

HD Trialfinder: A clinical trial matching resource for the North American HD community**George J. Yohrling**

Huntington's Disease Society of America

We have entered into a promising time for Huntington's disease (HD). With each passing day more pharmaceutical and biotechnology companies are entering into the HD drug development arena. While this is good news for all those affected by HD, recruitment of clinical trial participants in a timely manner is the greatest obstacle to developing the next HD treatment. In the past, if individuals were interested in trials, they were directed to resources such as Clinicaltrials.gov. Unfortunately, sites like this are outdated, difficult to understand and often do not provide a direct connection to the study site coordinator. All of these are unnecessary barriers to trial participation. HDSA identified the lack of a reliable clinical trial resource as a critical need in the HD community so we collaborated with EmergingMed to develop a new resource called HD Trialfinder. The HD Trialfinder, is an easy-to-use clinical trial matching service that connects individuals with HD, caregivers, healthy volunteers and physicians with current HD studies around North America. The HD Trialfinder is updated daily to ensure its database includes only those HD studies that are currently recruiting. The trial listings in the HD Trialfinder come from publicly available sources, such as clinicaltrials.gov. In addition, HDSA makes direct outreach to researchers and trial sites across the country to include their HD related clinical research studies that are not listed in clinicaltrials.gov. Trials not already listed in ClinicalTrials.gov are subject to additional review by the HDSA Scientific Advisory Board prior to being listed to ensure that listings only include credible trials and investigators. The HD Trialfinder only lists trials and studies that have Institutional Review Board (IRB) approval. To access HD Trialfinder, go to www.hdsa.org or www.HDTrialfinder.org. First-time visitors must create an account by providing an email address, and first name or alias. The program will allow you to create unlimited profiles for yourself, family, friends or your patients. To best match to a trial you must complete a brief questionnaire composed of 11 questions about yourself (or the HD impacted individual). HD Trialfinder will provide you with a patient-friendly summary of the different clinical studies, and the contact information for the nearest HD study coordinator to start the conversation about your eligibility. It is our hope that HD Trialfinder will be a valuable resource to the entire HD community and will work to expedite the recruitment of current and future HD trials.

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GROUP B: TRANSLATIONAL MEDICINE

Developing a huntingtin PET imaging tracer: in vitro and ex vivo assays to monitor binding of ligands to mutant huntingtin aggregates**Frank Herrmann**

Evotec

Huntington's disease (HD) is a neurodegenerative disease that would greatly benefit from the availability of a CNS PET ligand for quantification and assessment of mutant huntingtin (mHTT) aggregates in the HD patient's brain during HTT-lowering therapeutic trials. Here we present in vitro and ex vivo assays established to support development of such a PET ligand. Due to the difficulties associated with obtaining mHTT aggregates from HD brains, aggregates generated from recombinant purified mHTT protein and aggregates generated in preclinical HD in vitro and in vivo models were used for assay development. To detect ligand binding to these various types of aggregates and proteins, several types of in vitro and ex vivo systems were applied: (I) a higher throughput radioligand binding assay (RBA) utilizing recombinant aggregates and conventional filter-plate approach, (II) a radiometric filter trap assay (RAFTA) established to detect aggregate binding in more complex matrices such as cell lysates or brain homogenates, and (III) autoradiography (ARG) using HD mouse brain sections to monitor target engagement of selected radioligands against aggregates residing in a more physiological milieu. Using the RBA, we characterized various potential ligand series based on their competition profile with pre-determined tritium-labelled mHTT aggregate-binding ligands used as probes. In addition, this platform was used to characterize the properties of novel radioligands binding to the recombinant mHTT aggregates. Because natural HTT aggregates present in more complex matrices such as homogenates from cellular and animal HD models were not detected in the standard RBA format, we developed RAFTA, which makes use of membrane filters with smaller pore sizes than a conventional RBA filter plate. The combination of in vitro assays with ex vivo autoradiography binding assays have allowed the profiling and triaging of mHTT aggregate binders for their potential use as HD PET ligands. Future work will include further assessment of the ongoing chemical ligand development, as well as the utility and further development of these assays to characterize ligand binding to HD patient-derived mHTT aggregates.

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GROUP B: TRANSLATIONAL MEDICINE

Development of pre-clinical PET ligands for mutant huntingtin aggregates**Christopher Brown**

Evotec

Despite the initiation of huntingtin lowering clinical trials, biomarkers that can be used to assess target engagement and evaluate disease modification are currently lacking. A PET ligand that binds mutant HTT aggregates (mHTT) in both pre-clinical HD models and human subjects would allow changes in aggregate load in different brain regions over time to be monitored, and assist with translation from pre-clinical to clinical studies. An ideal PET ligand would penetrate the blood brain barrier and enter brain cells, bind mHTT aggregates with sufficient affinity to provide a signal over background noise, show little binding (signal) in non-diseased brain, be selective over other misfolded proteins, such as beta amyloid, and have suitable brain kinetics, metabolic profile and chemical structure for labelling with ¹¹C or ¹⁸F. Here we summarize our strategy to develop PET ligands directed to mHTT aggregates and highlight progress made during lead optimization. We will present data from a radioligand binding assay developed to detect binding to recombinant HTT aggregates, brain homogenate binding data that indicate low non-displaceable binding and highlight compounds with improved performance in autoradiography (ARG) using HD mouse brains. Binding selectivity data over beta amyloid binding in an ARG mouse AD model will be presented. In addition, we will show how translational analysis of in vivo and in vitro metabolite profiles can be used to design a PET labelling strategy with predicted minimal brain exposure of radiolabeled metabolites in humans. Finally, we will disclose results from mouse Q175 μ PET studies using novel PET ligands which may be suitable for pre-clinical HD studies.

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GROUP B: TRANSLATIONAL MEDICINE

Evaluation of six potential mutant huntingtin binding radioligands in non-human primate and in the zQ175 mouse model of Huntington's disease**Jenny Häggkvist**

Karolinska Institutet

Huntington's disease (HD) is caused by the expansion of CAG repeats in the huntingtin (HTT) gene. The CAG-expansion causes accumulation of mutant HTT protein (mHTT), which is associated with the loss of neurons leading to development of motor dysfunction, emotional disturbances, psychiatric symptoms and cognitive deficits. Today, there is strong evidence that lowering mHTT would be a potential therapeutic strategy for HD. Therefore, specific biomarkers for mHTT are of great value in order to be able to assess the efficacy of novel experimental therapies aimed at lowering mHTT. In the present study, we examined the properties of six new potential mHTT binding radioligands, [11C]CHDI-00481562, [11C]CHDI-00484210, [11C]CHDI-00483578, [11C]CHDI-00482879, [11C]CHDI-00482927 and [11C]CHDI-00485180 using positron emission tomography (PET) in healthy non-human primates (NHP) as well as in vitro autoradiography (ARG) and small animal PET imaging in the zQ175 knock-in mouse model (heterozygous and homozygous animals). All radioligands entered the brain in NHP as well as in WT and zQ175 mice. In the NHP, rapid kinetics and low non-specific binding was observed for [11C]CHDI-00484210, whereas [11C]CHDI-00481562 showed slow distribution to brain ($T_{max}=7.5$ min). The remaining tracers displayed comparable in vivo kinetic profiles in the NHP. In mice, all radioligands showed an initial uptake between 200-300 % SUV. [11C]CHDI-00484210 showed the most rapid washout from the brain and had also the lowest background binding in WT animals. All the other radioligands showed similar in vivo kinetics. The binding of the radioligands, as measured by % SUV and AUC, was lowest in WT, moderate in heterozygous zQ175 and highest in homozygous zQ175 animals. To compare the different radioligands, the AUC ratio between zQ175 and WT were calculated by dividing the average AUC for Het and Hom zQ175 respectively with the average AUC for the WT. [11C]CHDI-00485180 and [11C]CHDI-00482879 showed the highest binding as expressed by AUC, as well as the highest binding ratio both in heterozygous and homozygous animals. The results from the PET-imaging studies in rodents were confirmed in vitro by autoradiography. Taken together, this preclinical evaluation of [11C]CHDI-00481562, [11C]CHDI-00484210, [11C]CHDI-00483578, [11C]CHDI-00482879, [11C]CHDI-00482927 and [11C]CHDI-00485180 in NHP and in zQ175 mouse brain suggests that several of these radioligands are potential candidates for study as PET imaging agents in HD patients.

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GROUP B: TRANSLATIONAL MEDICINE

A virtual humanized computer model of neuronal brain network circuits in Huntington's disease**Geerts Hugo**

In Silico Biosciences

Evidence suggests that motor symptoms in Huntington's disease (HD) are driven by specific pathology in D2R Medium Spiny Neuron pathology in the indirect pathway of the basal ganglia region. Deep brain recording experiments in HD and Parkinson's disease (PD) patients suggest that the power spectrum of local field potentials in the subthalamic nucleus is associated with hyperkinetic and dyskinetic motor symptoms. We developed a mechanism-based complex Quantitative Systems Pharmacology (QSP) humanized computer model of the anatomically constrained interaction between motor cortical regions and various regions in basal ganglia of the dorsal motor circuit. This advanced computer model simulates the power spectrum of local field potentials in the subthalamic nucleus based on biophysical realistic representations of neurons firing action potentials derived from Hodgkins-Huxley equations. Conductances of voltage-gated ion channels are modulated by a large number of G-Protein coupled receptors that are implemented using based on preclinical information. The impact of medications is simulated using the proper target engagement level derived from human imaging studies. After implementing HD pathology (specific decline of D2+ MSN neurons) we tested the digital signature of various therapeutic interventions, such as tetrabenazine, antipsychotics, antidepressants, benzodiazepines and NMDA antagonists on the subthalamic nucleus local field oscillations. Cognitive impairment is an additional burden in HD patients. We have developed a QSP model with cognitive readouts that is calibrated using experimental human data on the N-back working memory test. The QSP model also includes five different human genotypes, based on mechanistic imaging studies. Unlike animal models, this 'hybrid' model while based on preclinical information is constrained by clinical data on human genotypes, imaging, pathology and therapeutic interventions and can be improved iteratively using feedback from predicted clinical outcomes. The platform 'formalizes' existing domain knowledge, allows simulating virtual HD patients in 'clinical trial' conditions for motor and cognitive readout and the impact of comedications and genotypes on the pharmacodynamic effect of new investigative compounds. In addition, it can be used for repurposing existing drugs, possibly offering a fast way to clinical development.

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GROUP B: TRANSLATIONAL MEDICINE

Therapeutic role of P42 peptide in Huntington's disease**Florence Maschat**
Montpellier University

Several studies, including ours, indicate an influence of the amount of normal hHtt on Huntington's disease (HD) severity. Our studies took advantage of a *Drosophila* model of HD, expressing human polyQ-Htt: we could rescue the polyQ-hHtt induced toxicity in different tissues by expressing around 600aa of the N-terminus part of human or *Drosophila* Htt (Mugat et al., HMG, 2008). Based on these results, we identified, within the normal Htt, a 23aa peptide (P42) able to inhibit different phenotypes, induced by the expression of the polyQ-hHtt (Arribat, Bonneaud et al., PlosONE, 2013). The protective properties of P42 have been also tested in R6/2 mice model of HD. To ensure brain-blood-barrier (BBB) crossing, we fused P42 to TAT, a protein transduction domain. Also, in order to perform repetitive delivery of the peptide, we tested lipidic transporters using water-in-oil microemulsion drug delivery vector termed Aonys® (Medesis-pharma), allowing oral/rectal transmucosal non-invasive administrations. Our data confirmed a beneficial contribution of P42 (Arribat et al., Acta-Neuropathologica comm., 2014). We identified that P42 is able to act at different levels: at early stages on aggregation process, but also at later stages on BDNF level of expression and neuronal activity. Finally P42 being a part of the Htt protein, we found that P42 is necessary for vesicular recruitment and transport along the microtubules. Therefore P42 presents a dual activity: by lowering the negative effect of the mutant polyQ-Htt, and by enhancing the normal function of the Htt. These observations suggest a peculiar therapeutic potential, which recently led to an orphan designation by the European Medicines Agency.

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GROUP B: TRANSLATIONAL MEDICINE

KMO inhibition in the conscious non-human primate: Kynurenine pathway metabolite and neurotransmitter release in the caudate nucleus and prefrontal cortex**Roger Cachope**
CHDI Management / CHDI Foundation

Metabolites of kynurenine pathway (KP) are implicated in the pathophysiology of neurodegenerative disorders including Huntington's disease (HD). Kynurenine 3- monooxygenase (KMO) inhibitors may have potential to treat HD, given that KMO inhibition should shift the metabolism of kynurenine to increase the formation of the neuroprotective metabolite kynurenic acid (KYNA) and reduce the neurotoxic metabolites 3-hydroxykynurenine (3-OH-KYN) and quinolinic acid (QA) in the brain. This has been confirmed in rodent microdialysis studies using the potent KMO inhibitor CHDI-00340246. Here, we studied the effects of CHDI-00340246 on level of KP metabolites, free compound and neurotransmitter release in the dorsal caudate nucleus and the dorsolateral prefrontal cortex (PFC - area 9) in conscious young adult male cynomolgus macaques using microdialysis. Oral administration of 10-30 mg/kg of CHDI-00340246 increased concentrations of KYNA and AA in both brain areas. Importantly, CHDI-00340246 reduced concentrations of the neurotoxic metabolites 3-OH-KYN and QA in both brain areas. The compound did not affect the neurotransmitters dopamine, serotonin, glutamate, glycine and GABA, except that dopamine concentrations were reduced in the PFC (highest dose only). These findings demonstrate that oral dosing of CHDI-00340246 modulates the pathway centrally in Non-Human Primates and may have neuroprotective potential in HD.

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GROUP B: TRANSLATIONAL MEDICINE

HD Brain-Train: Enhancing neural plasticity using real-time fMRI neurofeedback training**Sarah Tabrizi**

University College London

Huntington's disease (HD) is a genetic, neurodegenerative disorder that leads to extensive brain atrophy and is characterized by progressive motor impairment, cognitive decline and neuropsychiatric symptoms. It is well-established that prior to symptom manifestation, there is a long prodromal period characterised by marked atrophy in the striatum, accompanied by changes in neuronal function, despite the absence of overt clinical symptoms. This suggests that there is a mechanism of compensation that supports preserved behaviour, despite the onset of pathology even at pre-manifest stages of the disease. As disease pathology progresses, this mechanism may no longer be able to sustain intact performance and symptoms begin to manifest. Brain training could be used to support neurorestoration in neurological disorders both as a primary and as an adjunct treatment to other disease modifying therapies. In HD it could potentially support existing compensatory mechanisms and delay disease onset, act as a dose regulator to other disease modifying treatments or boost brain re-organisation and restore function following e.g. mutant HTT clearance. The present study is the first to use real-time fMRI (rt-fMRI) neurofeedback training, a form of brain training, in HD patients. Ten patients with very early stage disease were trained to regulate their own brain activity volitionally by receiving near real-time visual feedback about the level of brain activation in their premotor cortex (PMC). They were all able to successfully learn to increase their PMC activity over multiple training sessions. Grey matter density within the PMC, the trained area, also increased following training, suggesting the presence of training-related neuroplasticity. Critically, patients showed improved cognitive and motor capacity following training in several well-validated tests from the Track-HD assessment battery, including emotion recognition, circle tracing, symbol-digit matching test and finger tapping. Our results provide preliminary evidence that rt-fMRI neurofeedback training can be used to induce neural plasticity in HD with potentially positive effects on cognitive and motor function. We are currently conducting a randomized and controlled proof-of-concept study using a larger sample group to further evaluate the effects of neurofeedback training in HD. Because rt-fMRI neurofeedback training is non-invasive and low-risk, it can be readily used either preventatively or in conjunction with disease modifying therapies, such as gene-silencing, in order to further increase their effectiveness in restoring cognitive and motor function following treatment.

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GROUP B: TRANSLATIONAL MEDICINE

Harmonisation of a magnetic resonance spectroscopy acquisition sequence for use in clinical trials of Huntington's disease**Eileanoir Johnson**

IXICO

Magnetic Resonance Spectroscopy (MRS) is a technique used to measure the concentration of different metabolites in the brain, and can be used to detect biochemical brain changes caused by illness or disease. The current study aimed to develop harmonised MRS acquisition protocols suitable for use in multi-site clinical trials for Huntington's Disease (HD). The protocols were optimised on MRI scanners from three leading manufacturers: Philips, Siemens and General Electric Healthcare (GE). The primary focus was on achieving a reproducible longitudinal measurement, while a secondary objective was maximising the agreement of the measured metabolite concentration. The results of this study provide a guide for the design, set-up and analysis of longitudinal MRS multi-site studies and clinical trials. Three scanners located in London (UK) were included in this study; A Philips 3T Achieva scanner, a Siemens 3T TimTrio scanner and a GE 3T Discovery MR750 scanner. A Philips Point RESolved Spectroscopy (PRESS) MRS protocol previously used in a number of observational HD studies was modified for application on all three types of scanner. An iterative approach was used to harmonise the protocol between scanners, with a phantom scan conducted on each scanner followed by three iterations of healthy volunteer scanning. The same two volunteers were scanned at each site for all iterations. Voxels were placed in the putamen and frontal white matter. Due to restrictions on the GE scanner, voxels for all scanner types were aligned in parallel with the magnet axes. All spectra were analysed using LCModel software [version 6.3-01] to estimate the concentrations of a number of metabolites. There was a focus on two metabolites previously found to be sensitive to change in HD, N-acetyl-aspartate (NAA) and myo-inositol (mI). After all scanning was completed, the mean intra- and inter-session Coefficient of Variation (CoV) obtained for the metabolites of interest (NAA and mI) were found to be below 20% in general (both for the water referenced metabolite concentrations and in concentrations expressed as a ratio to Creatine). In particular, NAA CoVs were below 20% in all cases except for the mean inter-session CoV obtained from Siemens data in the frontal white matter. This study implemented standardized protocols across the three major scanner vendors for the acquisition of MRS data in the putamen and white matter. Protocols were developed with acceptable levels of within and between session variability for NAA and mI, two metabolites typically of interest in HD.

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GROUP B: TRANSLATIONAL MEDICINE

Evaluation of pridopidine in the transgenic YAC128 mouse model of Huntington disease**Marta Garcia-Miralles**

Translational Laboratory in Genetic Medicine

Pridopidine has been previously shown to improve measures of motor function in patients with Huntington disease (HD), although its mechanism of action is poorly understood. Here we aim to investigate the efficacy and mechanism of action of pridopidine using the transgenic YAC128 mouse model of HD. Pridopidine was administered to animals starting at early (1.5 months of age) or late stages of disease (8 months of age). In the early treatment cohort, animals were divided into three groups receiving 0, 10, or 30 mg/kg of pridopidine for a period of 10.5 months. In the late cohort, animals were divided into two groups receiving either 0 mg/kg or an escalating dose of pridopidine (10 mg/kg in week 1, 20 mg/kg in week 2, and 30 mg/kg in weeks 3-8). The treated animals were evaluated using a battery of behavioural tests. Preliminary analyses reveal that pridopidine treatment improves motor coordination and depressive-like phenotypes in the YAC128 HD mice. Neuropathological and molecular assessments to investigate the mechanism of action of pridopidine are currently underway. Our study supports continued clinical development of pridopidine for HD.

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GROUP B: TRANSLATIONAL MEDICINE

HDClarity: A new multi-site cerebrospinal fluid collection initiative to facilitate therapeutic development for Huntington's disease**Edward Wild**

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With several clinical trials in progress and more expected to launch in the next few years exploring novel therapeutic approaches for treating Huntington's disease, biomarkers are needed to evaluate target engagement, efficacy and disease progression.

Cerebrospinal fluid (CSF) is an ideal fluid compartment for assessing HD biomarkers, particularly pharmacodynamic markers, due to its proximity to the brain. There is currently no high quality repository of CSF from well-characterized HD gene expansion carriers spanning the disease spectrum. HDClarity will provide such a repository in order to expedite the research into biomarkers for HD.

In one usage, the sample collection will be assayed to determine if the kynurenine pathway (KP) is dysregulated in premanifest and early HD in comparison to healthy controls, and to evaluate the variability in KP metabolite levels within each participant group. The sample collection will also enable the further development and validation of assays to measure huntingtin protein (HTT) in CSF, an attractive pharmacodynamic biomarker for HTT lowering clinical trials. The sample collection will also enable the continued evaluation of a number of potential novel biomarkers of disease stage and progression. A CSF Consortium consisting of the CI, CHDI and Pls interested in research uses of CSF will provide scientific oversight into the use of samples and analysis of data.

CSF and blood samples will be collected at up to 30 sites using a standardized protocol. Careful collection of clinical and phenotypic data on each donor will enable us to appropriately select subsets of samples for each set of experimental assays. HDClarity uses the Enroll-HD Platform to identify participants and collect standardized data.

Six participant cohorts will be included in the study:

1. Healthy controls, n= 100
2. Early Pre-manifest HD, n=100
3. Late Pre-manifest HD, n=100
4. Early Manifest HD, n = 100
5. Moderate Manifest HD, n=100
6. Advanced Manifest HD, n=100

Participants will attend a Screening Visit, a Sampling Visit and an optional Repeat Sampling Visit (4-8 weeks after the Sampling Visit).

HDClarity is sponsored by University College London (UCL), coordinated at UCL Huntington's Disease Centre, and funded and supported by CHDI Foundation, Inc.

Investigators at prospective clinical sites with an active Enroll-HD cohort are invited to email the Chief Investigator (e.wild@ucl.ac.uk).

Co-authors: Beth Borowsky and the HDClarity investigators



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GROUP B: TRANSLATIONAL MEDICINE

Cerebrospinal fluid inflammatory biomarkers in Huntington's disease: an exploratory cross-sectional study**Edward Wild**

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INTRODUCTION Inflammation has been implicated in HD pathogenesis and biomarkers for this process could be relevant to better characterise the therapeutic response to specific interventions. The aim of this exploratory work was to study general inflammatory cytokines – TNF- α , IL-1 β , IL-6 and IL-8 – and microglial activation markers – YKL-40 and chitotriosidase – in the cerebrospinal fluid (CSF) of HD patients. **METHODS** We assayed CSF TNF- α , IL-1 β , IL-6, IL-8, YKL-40 and chitotriosidase from 23 mutation carriers, of whom 20 were symptomatic subjects, and 14 healthy controls. CSF TNF- α , IL-1 β , IL-6, IL-8 and YKL-40 were assayed using Meso Scale Discovery antibody-based assays with electrochemiluminescence detection tetra-plex kit. Chitotriosidase was measured using an in-house enzyme activity assay. **RESULTS** CSF TNF- α and IL-1 β were below the limit of detection. Mutation carriers had higher YKL-40 ($p=0.0028$), chitotriosidase ($p=0.0145$) and IL-6 ($p=0.0405$) than healthy controls. No molecule showed significant differences between presymptomatic and symptomatic subjects. YKL-40 showed significant association with age, and YKL-40 and IL-8 showed significant association with disease burden. YKL-40 significantly correlated with disease stage ($r=0.45$, $p=0.013$), total functional capacity score ($r=-0.46$, $p=0.015$), and UHDRS total motor score ($r=0.65$, $p=0.000$). After adjustment for age, the association with total motor score remained significant. No other significant correlation was observed. **CONCLUSIONS** CSF biomarkers of inflammation and microglial activation may be useful as biomarkers for HD. YKL-40 can predict clinical phenotype and may be a useful as a disease activity and pharmacokinetic biomarker. Further investigation is needed to support our exploratory findings. **FUNDING** CHDI Foundation Inc, GSK, Medical Research Council UK, Swedish Research Council and the Wolfson Foundation.

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GROUP B: TRANSLATIONAL MEDICINE

A LC-MS/MS method for the determination of biomarkers of oxidative stress status of DNA and proteins in human and C57BL/6 mouse urine**Pamela Di Pasquale, Simone Esposito**

IRBM Science Park

Oxidative stress (OS) has been associated with a number of diseases including cancer, arteriosclerosis and a range of neurodegenerative disorders which include Huntington's disease (HD). Increased OS within a biological system has been implicated in increased protein and DNA modification, increased inflammation and tissue damage and premature apoptosis in OS sensitive cells. Various treatments have been proposed and tested for reducing OS including exercise, antioxidant supplements and most recently, stimulators of the Nrf2-ARE pathway. The latter is of particular interest in that Nrf2-ARE stimulators attempt to reduce OS by increasing a cell's endogenous antioxidant capacity rather than through the dosing of an external antioxidant. It has been postulated for the current research that the identification of one or more OS biomarkers could prove to be a useful tool for the evaluation of HD progression or treatment efficacy. To this purpose, a study for the determination of OS biomarkers in several human and mouse matrices, including urine, plasma and tissues is currently ongoing. The study is divided into two main parts: 1) development and validation of analytical methods, and 2) analysis of study samples from HD patients and HD mouse models. The investigated biomarkers cover three different OS-related phenomena: protein damage (dityrosine, 3-nitrotyrosine, and 3-chlorotyrosine), DNA damage (8-hydroxy-deoxyguanosine), and lipid peroxidation (isoprostanes). In this poster, we detail the methods developed for the determination of these biomarkers in human and mouse urine. These methods are based on solid-phase extraction for the purification of the various biomarkers from the biomatrix, followed by quantification by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The developed methods allow the determination of dityrosine, 3-nitrotyrosine, 3-chlorotyrosine, 8-hydroxy-deoxyguanosine and selected isoprostanes in mouse and human urine at sub ng/mL levels. The developed bioanalytical methods have been validated or are in the process of validation using industry standard criteria to assure reproducibility of the methods when applied in laboratories across the HD community.

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GROUP B: TRANSLATIONAL MEDICINE

The role of the cerebellum in juvenile Huntington's disease**Peg Nopoulos**

University of Iowa

Objectives: The cerebellum is integrated structurally and functionally with the striatum. In particular, the motor portion of the cerebellum (anterior lobe) has input into the indirect pathway of the striatum, the pathway responsible for movement inhibition, and the pathway affected most by HD. Although in Adult Onset HD (AOHD) the cerebellum may be compensatory in nature, in JHD, hyper-connectivity of the cerebellum could lead to the hypokinetic state seen in JHD subjects. **Methods:** The University of Iowa Kids-JHD program enrolls children 8-18 years of age who have already received the clinical diagnosis. Each child undergoes an MRI scan. A total of 10 children with JHD, ages 6-17 were studied (mean CAG=77). JHD subjects were compared to a large data base (>200 subjects) of control children. Structural brain measures and DTI measures were evaluated between groups. **Results:** Subjects with JHD had substantially reduced Intracranial Volumes (ICV) which is a proxy measure of brain growth. After controlling for the overall small size of the brain, JHD subjects had reduced volumes of the cerebrum, mostly accounted for by a decrement in white matter. The cortex was completely normal in volume. The striatum (caudate and putamen) and globus pallidus were specifically affected with volumes that were far below normal. Thalamus and hippocampus were of normal size. Importantly, the cerebellum was enlarged in the JHD sample - this was most prominent in the anterior (motor) portion. DTI measures showed that the white matter tracts connecting the cerebellum to the striatum were stronger than normal indicating a hyper-connectivity between the cerebellum and the striatum. Finally, the size of the cerebellum and the strength of the associated white matter tracts were directly related to abnormalities in motor function (the larger the volume, the higher the FA in white matter tract, the worse the motor scores). This suggests that the enlargement and hyper-connected cerebellum is driving the hypokinetic state in the JHD subjects. **Interpretation.** HTT is vital for normal brain development. In its mutant form, mHTT may alter the development of the striatum which in turn spurs the development of the cerebellum to have greater input to the indirect pathway. In AOHD this is likely compensatory. However in JHD, the striatal development is so severe that the cerebellar compensation is driven so far that instead of facilitating function, it actually inhibits motor function, manifesting in the hypokinetic state of JHD.

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GROUP B: TRANSLATIONAL MEDICINE

Identification of CNS-penetrant Rho kinase inhibitors with improved kinase selectivity profiles towards proof of concept studies in animal models of Huntington's disease**Omar Aziz**

Charles River

Rho kinases (ROCK) belong to the serine-threonine family and are involved in a variety of functions, axonal growth, synapse stability and migration. Rho kinase inhibition has also been shown to be beneficial in dopamine receptor 2 driven striatal neuronal survival. ROCK 1 and 2 isoforms demonstrate high sequence similarity but differential tissue expression, with ROCK2 being more prominent in the CNS, whilst ROCK1 is more abundant in peripheral organs. Abnormal activation of ROCK and downstream effectors has been reported in several CNS disorders, and it has been postulated that abnormal ROCK activation might play a role in the pathology of Huntington's disease. Importantly, profilin (a downstream effector of ROCK) is reported to interact directly with huntingtin (Htt), and signaling components of the ROCK cascade are modifiers of mHtt aggregation. A number of ROCK inhibitors (ROCKi) exist including drugs such as fasudil and ripasudil for peripheral indications. Pharmacological ROCKi have been reported to have a benefit in neuronal degeneration, although no known ROCKi combine kinase selectivity with properties consistent with CNS exposure. We have identified potent ROCKi with improved kinase selectivity that exhibit good CNS exposure. These ROCKi have potential as tool compounds to assess whether reduction of mHtt aggregation in an HD model system has a positive impact on survival. Current efforts are targeted towards measurement of compound efficacy following in vivo dosing through a ROCK dependent mechanism. We will present different strategies towards the development of an ex vivo biomarker to evaluate the selective and CNS penetrant compounds that we have identified. **References** The therapeutic effects of Rho-ROCK inhibitors on CNS disorders. Kubo, T.; Yamaguchi, A.; Iwata, N.; Yamashita, T. *Ther. Clin. Risk. Mgmt.* 2008, 4(3), 605-615. Rho Kinase (ROCK) inhibitors and their therapeutic potential. Feng, Y.; LoGrasso, P. V.; Defert, O.; Li, R. *J. Med. Chem.* 2015, DOI: 10.1021/acs.jmedchem.5b00683 Rho kinase, a promising drug target for neurological disorders. Mueller, B. K.; Mack, H.; Teusch, N. *Nat Rev Drug Discov* 2005, 4, 387-398. Inhibition of rho kinase enhances survival of dopaminergic neurons and attenuates axonal loss in a mouse model of Parkinson's disease. Tönges, L.; Frank, T.; Tatenhorst, L.; et al. *Brain* 2012, 135, 3355-3370.

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GROUP B: TRANSLATIONAL MEDICINE

Mitochondrial fission and fusion in skeletal muscle from HD patients and zQ175 mice**Kerstin Kojer**

Ulm University

Objective: The aim of our study was to determine the steady state and dynamic fission and fusion balance in quadriceps muscle tissue of the zQ175 HD mouse model and human HTT gene expansion carriers. Background: In human HD, naturally occurring variability in genes relevant for mitochondrial quality control (MQC) was associated with motor age-at-onset (1). Mitochondrial fission and fusion is an important part of the MQC. In post-mortem brain tissue from HD patients as well as in human HD fibroblasts, mHTT interferes with Drp1, the major fission enzyme, leading to an increased fragmented mitochondrial network. In neurons, mHTT-mediated mitochondrial fragmentation and defects in mitochondrial transport can be rescued by reducing the Drp1 activity (2, 3). Material and methods: mRNA expression and protein analyses in quadriceps tissue of zQ175 mice (6 months and 9 months) and in near to motor onset pre-manifest HD (n=20), early motor onset HD patients (n=20), and sex and age matched healthy controls (n=20), as part of the Multiple-Tissue Molecular Signatures in HD project (MTM-HD). In addition, we analyzed the mitochondrial network in human primary fibroblasts and myoblasts after life cell staining with Mitotracker Green. Results: Steady state tissue Drp1 mRNA levels were decreased in early HD patients and zQ175 mice. In addition Drp1 protein levels were lower in early HD patients compared to controls. Drp1 is localized in the cytoplasm and is recruited to the mitochondrial outer membrane to induce fission. We did not observe differences in Drp1 levels in mitochondrial subcellular fractions. However, there was a slight increase in phosphorylated (Ser 616) Drp1 in zQ175 mice. Furthermore, in human primary HD fibroblasts and myoblasts the mitochondrial network was phenomenologically similar to control cells. Conclusions: We found no evidence for a disruption of the steady-state fission-fusion balance in skeletal muscle of zQ175 mice or human HD gene carriers. The presence of mHTT alone does not compromise the steady-state fission and fusion balance at least at the ages of zQ175 mice and stages of human HD that were examined. References: 1) Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium Cell. 2015 Jul 30;162(3):516-26 2) Shirendeb et al. Hum Mol Genet. 2012 Jan 15;21(2):406-20 3) Song et al. Nat Med. 2011 Mar;17(3):377-82

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GROUP B: TRANSLATIONAL MEDICINE

CYP46A1, the rate-limiting enzyme for cholesterol degradation, is neuroprotective in Huntington's disease**Nathalie Cartier**

INSERM

Huntington's disease is an autosomal dominant neurodegenerative disease caused by abnormal polyglutamine expansion in huntingtin (Exp-HTT) leading to degeneration of striatal neurons. Altered brain cholesterol homeostasis has been implicated in Huntington's disease, with increased accumulation of cholesterol in striatal neurons yet reduced levels of cholesterol metabolic precursors. To elucidate these two seemingly opposing dysregulations, we investigated the expression of cholesterol 24-hydroxylase (CYP46A1), the neuronal-specific and rate-limiting enzyme for cholesterol conversion to 24S-hydroxycholesterol (24S-OHC). CYP46A1 protein levels were decreased in the putamen, but not cerebral cortex samples, of post-mortem Huntington's disease patients when compared to controls. Cyp46A1 mRNA and CYP46A1 protein levels were also decreased in the striatum of the R6/2 Huntington's disease mouse model and in SThdhQ111 cell lines. In vivo, in a wild-type context, knocking down CYP46A1 expression in the striatum, via an adeno-associated virus-mediated delivery of selective shCYP46A1, reproduced the Huntington's disease phenotype, with spontaneous striatal neuron degeneration and motor deficits, as assessed by rotarod. In vitro, CYP46A1 restoration protected SThdhQ111 and Exp-HTT-expressing striatal neurons in culture from cell death. In the R6/2 Huntington's disease mouse model, adeno-associated virus-mediated delivery of CYP46A1 into the striatum decreased neuronal atrophy, decreased the number, intensity level and size of Exp-HTT aggregates and improved motor deficits, as assessed by rotarod and clasp behavioural tests. Adeno-associated virus-CYP46A1 infection in R6/2 mice also restored levels of cholesterol and lanosterol and increased levels of desmosterol. In vitro, lanosterol and desmosterol were found to protect striatal neurons expressing Exp-HTT from death. We conclude that restoring CYP46A1 activity in the striatum promises a new therapeutic approach in Huntington's disease.

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