A Pathogenic mechanisms

A.1 DISENTANGLING MOLECULAR INTERACTION NETWORKS FOR CHOREA HUNTINGTON

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Background: Although chorea Huntington is a classic Mendelian disease following dominant inheritance pattern, strong interindividual variability in the disease progression suggests the existence of biological modifiers that could provide novel therapeutic targets.
Aims: Our approach is directed towards the detection of novel disease modifiers and consolidation of the numerous seemingly unrelated molecular changes observed during chorea Huntington progression.
Methods: A htt-focused protein interaction network was constructed to capture the molecular context of Huntingtin. For the identification of modifiers, a novel multi-level prioritisation strategy based on complementary information was developed.
Results: Using this network approach, we were able to identify a set of potential modifiers. One of the identified modifiers was subsequently experimentally validated as an important factor for aggregation, neurotoxicity and disease progression in Huntington's disease models.
Conclusions: Our study demonstrates that network approaches can greatly facilitate the elucidation of the molecular mechanisms underlying chorea Huntington.

A.2 LOSS OF HUNTINGTIN INTERACTING PROTEIN HIP14 IN VIVO RECAPITULATES FEATURES OF HUNTINGTON'S DISEASE

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HIP14 (ZDHHC17) is a mammalian palmitoyl transferase that interacts less robustly with mutant polyglutamine expanded htt compared with wild type (wt). As a result, the palmitoylation of mutant htt is significantly reduced. If altered palmitoylation of mutant htt by HIP14 is crucial in the pathogenesis of Huntington's disease (HD), HIP14–/– mice might be expected to display a phenotype similar to HD. Indeed, HIP14–/– mice displayed many similar features to the HD phenotype in YAC128 htt transgenic mice. These included a significant and specific decrease in striatal volume (wt: 13.5 ± 0.7, HIP14–/–: $11.2 \pm 0.8\ mm^3$, $n = 7$, $p = 0.001$), decreases in striatal neuronal counts (wt: $17.6 \pm 1.2 \times 10^5$, HIP 14−/−: $16.2 \pm 1.4 \times 10^5$, $n = 7$, $p = 0.08$) and size. In addition, HIP14−/− mice were defective in tests of motor coordination such as fixed and accelerating rotarod and swim speed. The mice also showed reduced sensorimotor gating as assessed by prepulse inhibition, all defects observed in the YAC128 HD mice. In addition, HIP14−/− mice displayed reduced corticostriatal transmission and significantly decreased presynaptic probability of release.

In contrast to YAC mice, an earlier, more severe phenotype was observed in the HIP14−/− mice, with specific striatal volume loss at 1 month rather than at 8 months in the YAC128 mice. In addition to htt, HIP14 palmitoylates several other proteins involved in synaptic function. One possibility for the enhanced phenotype is altered palmitoylation of other neuronal proteins identified as substrates of HIP14. Indeed, palmitoylation of post-synaptic proteins psd95, GluR1 and GluR2 and the presynaptic protein SNAP25 are reduced in HIP14−/− brains and localisation of both psd95 and GluR1 are altered in striatal neurons isolated from HIP14−/− mice.

Palmitoylation of proteins is essential for their trafficking to and turnover at specific plasma membrane sites. Altered localisation and turnover of glutamate receptors caused by aberrant palmitoylation may thus underlie the striatal loss in HIP14−/− mice. The similarity in phenotype between HIP14−/− and HD mice indicates that palmitoylation may play an essential role in the excitotoxic cell death pathway in HD.

A.3 PROTEIN–PROTEIN INTERACTIONS IN POLY-Q DISEASE MODEL

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Background: In disease models for Huntington's disease, modified Huntingtin (Htt) exon 1 with extended poly-Q stretches may confer neuroprotection under certain conditions. This may be due to conformational changes in Htt, exposure of the proline-rich region and changed protein–protein interactions, but the mechanism of this interesting phenomenon is not understood.
Aims: The aim of this project is to map the molecular details of these changed protein–protein interactions. We will investigate the difference in conformation between wild-type (wt) Htt and mutant variants and interactions with other proteins and wt and mutant Htt, especially with two chaperone heat shock proteins, which recently were found to be efficient suppressors of Htt aggregation.
Methods: We will use purified recombinant proteins and a combination of chemical crosslinking and mass spectrometry, a method recently developed by us.[1] Nuclear magnetic resonance, electron microscopy and *x* ray crystallography will also be used.
Results: The approach with chemical crosslinking and mass spectrometric mapping of crosslinked peptides for protein–protein interaction studies has been developed using a model system. Protein–protein interactions have been analysed within an oligomeric heat shock protein, Hsp21, and between Hsp21 and various substrate proteins in need of being protected from aggregation. We have verified previously suggested interactions between the subunit–subunit interfaces within the Hsp21 oligomer as a proof-of-principle of our experimental approach. Moreover, our data show that the Hsp21 monomer subunit interfaces become substrate binding surfaces upon oligomer disassembly.
Conclusion: Our approach will be useful for gaining insight into protein–protein interactions between Htt and suppressors of Htt aggregation.

1. **Åhrman E**, Lambert W, Aquilina A, *et al*. Chemical cross-linking of chloroplast localized small heat-shock protein, Hsp21, and the model substrate citrate synthase. *Protein Science* 2007;**16**:1464–87.

A.4 ACTIVATION OF CASPASE-6 IS AN EARLY EVENT IN ACUTE AND CHRONIC MODELS OF HUNTINGTON'S DISEASE

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Background: The characterisation of YAC mice expressing caspase-6-resistant (C6R) mutant huntingtin highlights proteolysis of htt at the 586aa caspase-6 (C6) site as a key mechanism and implicates activation of C6 as an early event in the pathogenesis of Huntington's disease (HD). The aim of this study was to determine

whether activation of C6 is observed in acute and chronic models of HD.

Results: We demonstrate a significant increase in C6 activity and cell death in primary striatal neurons expressing mhtt post *N*-methyl-D-aspartate treatment compared with untreated neurons. Pretreatment with a C6 inhibitor before *N*-methyl-D-aspartate rescues the cell death observed. In order to determine the time course of C6 activation in vivo we performed a natural history characterisation of C6 in wild-type (wt), YAC128 and C6R brain. Immunohistochemical analysis of wt brain demonstrates that the activation of C6 occurs predominantly in striatal neurons commencing at 9 months with an increase at 18 months. In contrast, striatal neurons in YAC128 brain display activation of C6 by 3 months, with levels increasing with age. Low levels of activated C6 are observed at 3 months in C6R striatum; however, levels remain constant with age. Assessment of C6 activity levels, using fluorogenic activity assays, confirms the immunohistochemical findings and demonstrates increased C6 activity in YAC128 versus wt striatum at 3 months (**$p<0.01$). In contrast, whereas cortical C6 activity is not increased in YAC128 at 3 months, by 12 months C6 activity is increased in YAC128 cortex compared with control (*$p<0.05$). In human brain tissue, increased levels of activated C6 are observed in presymptomatic and early grade HD striatum (**$p<0.01$) and grade 3–4 HD cortex (**$p<0.01$) compared with control brain.

Conclusion: These data further support the hypothesis that C6 is the protease responsible for the cleavage of htt at aa586 and demonstrate that activation of C6 is an early marker of neuronal dysfunction in the YAC128 model of HD.

A.5 DECREASING MUTATED HUNTINGTIN AGGREGATION AND TOXICITY BY OVEREXPRESSING MOLECULAR CHAPERONES OF THE DNAJ AND HSPB FAMILIES

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Background: Molecular chaperones (HspB, HspA and DNAJ families) together with degradation systems (proteasome and autophagy) allow cells to cope with misfolded and genetically mutated proteins. Some molecular chaperones have been shown to protect against protein aggregation and toxicity in polyglutamine (polyQ) diseases, but effectivity in vivo had remained limited so far.

Aims: We screened the chaperone activity of the small Hsp (HspB) and Hsp70 (HspA)/Hsp40 (DNAJ) family members in order to identify the most potent suppressors of polyQ aggregation and toxicity. We next focussed on similarities and diversities in their mechanism of action.

Methods/Techniques: Chaperone activity was tested in mammalian cell cultures using the filter trap assay technique and using in-vivo models of Huntington's disease (Xenopus) or SCA3 disease (*Drosophila melanogaster*).

Results/Outcome: Among the HspB family, we identified three members able to reduce polyQ aggregation, namely HspB7, HspB8 and HspB9. HspB8 works in concert with the co-chaperone Bag3 and stimulates autophagy, thus facilitating mutated huntingtin degradation. HspB7 is highly efficient in decreasing the aggregation rate of both short (43Q and 74Q) and long-length huntingtin (119Q). However, the mechanism is still unknown. HspB9 is mainly active on short-length polyQ proteins, by targeting them to the proteasome. Among the DNAJ family, a subfamily of the DNAJB chaperones, in particular DNAJB6 and DNAJB8 show the strongest chaperone activity against both short and long-length polyQ proteins. These chaperones appear to be regulated by (de)acetylation and to prevent aggregation as oligomeric complexes for which interaction with HspA is not required. Subsequent proteasomal degradation of DnaJB6 or DnaJB8-associated polyQ proteins, however, does rely on interaction with HspA members.

Conclusions: We identified several molecular chaperones that decrease polyQ aggregation by different mechanisms. The combined stimulation of these chaperones may act synergistically in clearing disease-associated proteins.

A.6 EFFECTS OF PARKIN DEFICIENCY ON MUTANT HUNTINGTIN IN MICE

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Huntington's disease (HD) is a neurodegenerative disorder caused by an increased expansion of polyglutamines in huntingtin, a protein that aggregates in the brain of patients with HD and in mice models of the disease. We investigated whether additional stress to the ubiquitin proteasomal system by partial suppression of the E3 ligase, parkin, changes disease severity. We crossed R6/1 mice with homozygous parkin null mice and produced four kinds of mice: HD+/−, HD+/−/PK+/−, wild type (wt) and PK+/−. HD+/− and HD+/−/PK+/− mice had abnormal behaviour, worse in the double mutant actitrack, labyrinth and length of stride.

HD+/− and HD+/−/PK+/− mice had decreased levels of monoamines and their metabolites in comparison with wt and PK+/− mice. The levels of reduced glutathione were increased in HD+/− and slightly more in the striatum of HD+/−/PK+/− mice. HD+/− and HD+/−/PK+/− mice had increased levels of apoptosis in the striatum, slightly greater in the double mutants. Huntingtin inclusions were present in HD+/− and HD+/−/PK+/− mice, less abundant in the double mutants. No differences were present in other regions.

Our data suggest that the suppression of parkin increases the pathogenic effects of mutant huntingtin more in the striatum than in other brain areas. Malfunction of the ubiquitin proteasomal system by mutant huntingtin appears as an additional but not critical mechanism of its toxicity.

A.7 THE USE OF QUANTITATIVE REAL-TIME PCR TO CHARACTERISE TRANSCRIPTIONAL DYSREGULATION IN MOUSE MODELS OF HUNTINGTON'S DISEASE

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Background: Transcriptional dysregulation is a central pathogenic mechanism in Huntington's disease (HD) and mouse models recapitulate the transcriptional changes that occur in the human disease. Quantitative real-time PCR (QPCR) assays that measure the transcript levels of genes for which transcriptional dysregulation is well established in HD could be used as biomarkers of disease progression in preclinical efficacy trials.

Aims: To establish a robust and accurate QPCR procedure using suitable reference genes that may be used to identify changes in gene expression in the R6/2 HD mouse model. To establish a test set of genes that can be used for preclinical assessment.

Methods: Reverse transcriptase QPCR and 2-$\triangle\triangle$Ct analysis was used to determine a set of reference and target genes for the cerebellum, cortex and striatum that identify expression changes in the R6/2 model compared with wild-type controls over time.

Results: Suitable housekeeping genes were ascertained for each region using the GeNorm software. These were used as reference genes for the normalisation of genes of interest. For each of the three brain regions a set of genes was identified that showed

transcriptional dysregulation. The gene expression was then characterised at 2, 4, 8, 12 and 15 weeks, showing that genes become increasingly dysregulated in the older R6/2 mice.
Conclusions: Reliable normalisation using appropriate housekeeping genes is essential for the detection of gene-specific variation using QPCR. Transcriptional profiles alter according to brain region over time and therefore have the potential to be used as biomarkers for the progression of HD.
Funding: This study received financial support from the Wellcome Trust, CHDI Foundation.

A.8 A LARGE NUMBER OF PROTEIN EXPRESSION CHANGES IN A MOUSE MODEL OF HUNTINGTON'S DISEASE EARLY IN LIFE PRECEDE OVERT DISEASE SYMPTOMS

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Background: Huntington's disease (HD) is fatal in humans within 15 to 20 years of symptomatic disease. Although late-stage HD has been studied extensively, data on protein expression early and during disease progression are scarce. In this study, we used a large 2-D gel/mass spectrometry-based proteomics approach to investigate HD-induced protein expression alterations and their kinetics at early ages and during the course of the disease.
Aims: Early protein changes and their progression in HD were studied.
Results and Outcome: The murine HD model R6/2 was investigated at 2, 4, 6, 8 and 12 weeks of age, corresponding to absent, early, intermediate and late-stage HD. Unexpectedly, there were mostly HD stage-specific protein changes (71%–91%) and a drastic alteration (6% of the proteome) in protein expression as early as 2 weeks of age. Early changes included mainly upregulation of glycolysis/gluconeogenesis and downregulation of actin-skeleton. This suggests a period of highly variable protein expression that precedes the visible HD phenotype. Whereas an upregulation of glycolysis/gluconeogenesis-related protein alterations remained dominant during HD progression, late-stage alterations at 12 weeks showed an upregulation of proteins having proteasomal function. The early changes coincide with a peak in protein alteration during normal mouse development at 2 weeks of age. Co-regulation between altered messenger RNA and protein expression is always below 30%, although 88% of the altered proteins were represented by mRNA.
Conclusions: Our observations suggest that HD is characterised by a highly dynamic disease pathology not represented by linear protein concentration alterations over the course of the disease.

A.9 NRF2-RELATED OXIDATIVE STRESS RESPONSE AND IMPAIRED DOPAMINE BIOSYNTHESIS IN A PC12 CELL MODEL OF HUNTINGTON'S DISEASE

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Background: Huntington's disease (HD) is a devastating disease for which currently no therapy is available. It is a progressive autosomal dominant neurodegenerative disorder that is caused by a CAG repeat expansion in the HD gene, resulting in an expansion of polyglutamines at the N-terminal end of the encoded protein, designated huntingtin, and the accumulation of cytoplasmic and nuclear aggregates. Not only is there a loss of normal huntingtin function, upon expansion of the CAG repeat there is also a gain of toxic function of the huntingtin protein and this affects a wide range of cellular processes.
Aims: To identify groups of genes that could play a role in the pathology of HD.
Methods: Messenger RNA changes were studied in an inducible PC12 model of HD before and after aggregates became visible.
Conclusions: This is the first study to show the involvement Nrf2-responsive genes in the oxidative stress response in HD. Oxidative stress-related transcripts were altered in expression suggesting a protective response in cells expressing mutant huntingtin at an early stage of cellular pathology. Furthermore, there was a downregulation of catecholamine biosynthesis resulting in lower dopamine levels in culture. Our results further demonstrate an early impairment of transcription, the cytoskeleton, ion channels and receptors. Given the pathogenic impact of oxidative stress and neuroinflammation, the Nrf2-ARE signalling pathway is an attractive therapeutic target for neurodegenerative diseases.

A.10 IDENTIFICATION AND CHARACTERISATION OF MICROGLIA-SPECIFIC SUPPRESSORS OF MUTANT HUNTINGTIN TOXICITY

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A number of studies have suggested a role for microglia in the onset and progression of Huntington's disease (HD). We have characterised the enzyme kyneurenine-3-monoxygenase (KMO), which is expressed predominantly in microglia in the central nervous system, as a candidate therapeutic target for HD by genetic approaches in yeast and pharmacologically in mammalian cells and in the R6/2 mouse model of HD. These studies suggest a role for microglia in the pathology of HD and also imply that cell autonomous effects of mutant huntingtin (htt) expression in microglia may contribute to HD pathology. Excitingly, recent studies in humans have shown a correlation between microglial activation and HD progression. We are therefore working to identify further microglia-specific therapeutic targets.

We are currently using a yeast model of mutant htt toxicity to screen complementary DNA libraries from unactivated, activated, and primary microglia to identify cDNA capable of suppressing mutant htt toxicity when overexpressed in yeast. We have identified 31 unique mouse cDNA capable of suppressing the toxicity associated with the expression of mutant htt in yeast. Many of these suppressors are associated with processes known to be affected in HD, including autophagy, protein folding, iron metabolism, actin cytoskeleton organisation and energy metabolism. The overexpression of a number of the suppressors has also previously been shown to be beneficial in a number of neurodegenerative disease models. Promising hits are being validated in a microglia–neuron co-culture system. We are also examining the expression patterns of these genes in microglia and other central nervous system cell types. Using these approaches we hope to gain insights into the microglia-specific cellular pathways that contribute to the pathology of HD. The screen may also aid in the identification of processes and specific proteins for therapeutic targeting in microglia and other cell types.

A.11 A NOVEL PATHOGENIC PATHWAY OF IMMUNE ACTIVATION DETECTABLE BEFORE CLINICAL ONSET IN HUNTINGTON'S DISEASE

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Background: Huntingtin is expressed ubiquitously and Huntington's disease (HD) can cause several abnormalities outside the central nervous system. We previously demonstrated evidence of immune activation in peripheral plasma in manifest HD using proteomic profiling, but the nature of the immune activation in HD remains incompletely explored.
Aims and Methods: To investigate inflammatory activation in HD, we used multiplex ELISA to quantify levels of key inflammatory and immunomodulatory molecules in human plasma and serum from three different mouse models of HD. We used quantitative real-time PCR to examine huntingtin expression in HD monocytes and cytokines in HD striatum and performed functional studies of HD monocytes, macrophages and microglia.
Results: We found widespread evidence of innate immune activation detectable in plasma throughout the course of HD. IL-6 levels were increased in HD gene carriers with a mean of 16 years before the predicted onset of clinical manifestations. To our knowledge, this is the earliest plasma abnormality identified in HD. Monocytes from HD patients expressed mutant huntingtin and were pathologically hyperactive in response to stimulation, suggesting that the mutant protein triggers a cell-autonomous immune activation. A similar pattern was seen in macrophages and microglia from HD mouse models and the cerebrospinal fluid and striatum of HD patients exhibited abnormal immune activation, suggesting that immune dysfunction plays a role in brain pathology.
Conclusions: Together our data suggest parallel central nervous system and peripheral pathogenic pathways of immune activation in HD.

A.12 HUNTINGTON'S DISEASE-RELATED MITOCHONDRIAL TOXINS AFFECT THE IMMUNOLOGICAL PROFILE OF MICROGLIAL CELLS

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Background: Mitochondrial dysfunction and microglia activation are both integral parts of Huntington's disease (HD). Mitochondrial toxins such as 3-nitropropionic acid (3-NP) and malonate induce HD-like degeneration in animals. These toxins as well as mutant huntingtin affect not only neurons but also microglia. Metabolic regulation and inflammatory responses are highly integrated and interdependent. The specific hypothesis is that mitochondrial alterations induced by toxins or mutant huntingtin in microglial cells change their immunological profile with detrimental consequences for the diseased brain.
Aim: We will investigate the effect of mitochondrial toxins on the immunological profile of microglial cells.
Methods: We incubated primary mouse microglia with the mitochondrial toxins 3-NP and malonate and investigated the effect of the mitochondrial toxins on cell viability (LDH assay) and mitochondrial respiratory chain activity (WST-1). To investigate the effect of mitochondrial toxins on the classic activation of microglial cells we stimulated microglial cells together with the toxins with 1 μg/ml lipopolysaccharide and/or 100 U IFN-γ. The amount of TNF-α, IL-1β, IL-6 and glutamate was quantified in the supernatant and/or in the cells after overnight incubation with adequate assays, as described in the manuals. An alternative activation was induced by stimulation with 10 ng/ml IL-4 for 24 h. The amount of cytokines was quantified as described.
Results: Mitochondrial toxins induce a time and dose-dependent decrease in mitochondrial respiratory chain activity, with subsequent cell death. The mitochondrial toxins changed the amount of classic and alternative induced release of cytokines and glutamate.
Conclusion: Our results show that changes in the mitochondrial respiratory chain activity can influence the immunological profile of microglial cells.

A.13 MAPPING MOLECULAR CHANGES IN HUNTINGTON'S DISEASE BRAIN

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With personal interests in systems biology and immunology, I shall attempt with this poster to map the various network connections/signalling pathways of at least some of the bioactive molecules that contribute either to neurodegeneration (nerve cell death) or to neuroprotection (nerve cell survival) in Huntington's disease (HD). In addition, HD appears to be a useful paradigm, or blueprint, for understanding the complex processes involved in other neurodegenerative conditions, such as Alzheimer's disease and Parkinson's disease. All three of these conditions exhibit neuroinflammation, a term that describes the effects on nerve cells of changes in the activation status of immune system cells present in the brain, notably microglia. Neuroinflammation is set off by definite trigger factors and this raises the question as to whether ''classic'' immune responses are generated in the brain in HD, as is clearly the case in multiple sclerosis. Some advantages of trying to build network models of HD are: (1) that it has a known single abnormal gene starting point; (2) there are good animal models of the disease; (3) at any one time, the ''system'' (illness), although very complicated, is essentially in a (pseudo)steady state and (4) it might provide one way of exploring the question: ''What are the immunological consequences of HD—and do they matter?''

A.14 DISTRIBUTION OF POLYGLUTAMINE INCLUSIONS IN NON-CENTRAL NERVOUS SYSTEM TISSUE IN THE HDHQ150 KNOCK-IN MOUSE MODEL OF HUNTINGTON'S DISEASE

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Background: We have previously conducted a phenotypic comparison of the R6/2 and HdhQ150 knock-in Huntington's disease (HD) mouse models. We found that when CAG repeat size, strain background and stage of disease are comparable both models develop similar widespread phenotypes. The distribution of polyglutamine aggregates throughout the central nervous system (CNS) was ubiquitous in both models.
Aim: To determine whether the polyglutamine aggregates are present in non-CNS tissues of the HdhQ150 mice and if so to compare the distribution in peripheral tissues with that previously established for R6/2 mice.
Methods: 22-month-old HdhQ150 homozygote knock-in and wild-type control mice were perfused with 4% paraformaldehyde. Organ and tissue samples were removed and processed for immunohistochemistry. Wax sections were immunoperoxidase stained with huntingtin antibody S830, counterstained with the nuclear stain methyl green and examined under the light microscope for the presence of huntingtin inclusions.

Results: Inclusions have been identified in the following peripheral organs in HdhQ150 knock-in mice: skeletal muscle, adrenal glands, liver, pancreas, kidney, the myenteric plexus and Meissner's plexus. This is similar to the pattern of aggregate distribution that we previously described in R6/2 mice.
Conclusions: The presence of inclusions in non-CNS tissues of R6/2 mice has not occurred because these mice express an N-terminal fragment of huntingtin as they are also present in the HdhQ150 knock-in model. It is not known whether a similar pathology occurs in the human disease but it would be expected to be a feature of childhood onset HD caused by large CAG expansions.
Funding: This study was supported by the Wellcome Trust and Medical Research Council.

A.15 EXPRESSION OF WILD-TYPE HUNTINGTIN IN PORCINE TESTICULAR GERM CELLS

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Background: Huntington's disease (HD) is a fatal dominantly inherited neurodegenerative disease caused by expansion of the polyCAG stretch in the gene coding ubiquitous huntingtin protein (htt). Despite the ubiquitous presence of the HD gene in a variety of organs/tissues, the HD-associated pathology is predominantly expressed in specific brain regions. In addition, more recent data indicate that the mutated htt can be linked directly with a progressive degeneration of germinal epithelium in the testes. *DAZL* (deleted in azoospermia-like) is an autosomal gene from the DAZ gene family that plays an important role in germ cell development. The absence of *DAZL* is manifested by a lack of germ cells with similar pattern to the testicular degeneration observed in HD.
Aims: The aim of this study was to characterise the expression of wild-type htt in the porcine testicular tissue and to develop a readily accessible in-vivo assay (in addition to brain neuronal pathology), which would have a predictable value in defining the stages of HD progression.
Methods: Tissue expression of htt was measured by Western blotting and coupled with immunofluorescence staining of paraformaldehyde-fixed frozen sections prepared from porcine testes using anti-htt (mouse monoclonal (HDA3E10) to Huntingtin, Abcam) and anti-*DAZL* (rabbit polyclonal to *DAZL*, Abcam) primary antibodies.
Results: Western blot analysis revealed htt expression in the testes. Immunofluorescence staining confirms a co-localisation of *DAZL* and wild-type huntingtin. In addition to the expression in the testes, the htt protein was measured in the cortex by Western blot.
Conclusions: Our preliminary data proved the physiological co-localisation of htt and *DAZL* in germinal epithelium. This indicates that the expression of mutated htt in *DAZL*+ in germinal epithelium can play a key role in the testicular degeneration seen in HD patients and can have prognostic value in characterising the stages of HD.

A.16 GENERATION AND EXPRESSION CHARACTERISATION OF BAC-HD TRANSGENIC RATS WITH FULL-LENGTH MUTANT HUNTINGTIN

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Our group has previously generated and characterised transgenic rats, which express a fragment of mutant huntingtin (htt), as a model of Huntington's disease (HD) in detail. This rat model mirrors many aspects of HD, but it lacks the full-length mutant htt protein and therefore some aspects of the human condition might be imperfectly replicated. In order to overcome this potential disadvantage, we aim to generate transgenic rats, which express full-length mutant human htt in the same developmental and tissue and cell-specific manner seen in patients with the disease. To achieve this, bacterial artificial chromosomes (BAC) containing human genomic DNA spanning the full- length gene including all regulatory elements are used, which have been successfully integrated in mice by Dr William Yang's laboratory.

We have established one rat line, in which full-length htt expression was detected in all the brain regions at a low level. The processing of the mutant htt and its aggregate formation are screened in this line. To obtain lines that express mutant htt at a higher level and in the brain areas affected in human HD patients, we generated 24 new founders, which show germline transmission by PCR. These founders are analysed for copy number of BAC insertion, integrity of the BAC insertion and quantity of CAG repeats. In F1 transgenic rats of each line the level of RNA and protein expression in their brains will be measured and the number of intergration sites will be identifed.

A.17 VALIDATION OF CANDIDATE THERAPEUTIC TARGETS FOR HUNTINGTON'S DISEASE IN DROSOPHILA

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We recently identified 28 gene deletions that suppress toxicity of a mutant huntingtin (htt) fragment in a yeast model of Huntington's disease (HD). Here we describe work validating a subset of these loss-of-function suppressors that have human homologues using a drosophila model of HD. In our experiments, we are directing mutant htt exon 1 93Q transgene expression both temporally and spatially via three promoters: (1) elav—which directs expression in most neurons; (2) gmr—which drives expression in all cells of the eye, including neurons and supporting cells and (3) tim—which is expressed in circadian clock neurons. The suppressors are being tested either via known loss-of-function alleles or using RNAi transgenic fly lines from the Vienna Drosophila RNAi Center. One of these suppressors being tested encodes the fly homologue of the mammalian enzyme kynurenine 3-monooxygenase (KMO). We have validated our initial yeast work pharmacologically in both mammalian cells and in the R6/2 mouse model of HD, making KMO a promising candidate therapeutic target for this disease. Here we report that a loss-of-function allele of the drosophila Kmo homologue, cinnabar (cn3), significantly enhances the number of rhabdomeres (eye photoreceptors) in Htt93Q cn3 individuals compared with Htt93Q expressing control flies, which suffer extensive eye degeneration. This work provides the first genetic evidence that inhibition of KMO function is neuroprotective in an animal model of HD and supports the notion of validating yeast candidate therapeutic targets in drosophila.

In addition to previously used metrics, we are studying behavioural phenotypes in HD model flies, such as circadian locomotor rhythms and visual tracking. We have examined circadian behaviour of the HD model flies that have been aged for several days, in light–dark cycles and constant darkness. We have observed that pan-neuronal expression of htt using the elav promoter led to reduced levels of locomotor behaviour in the flies and an impaired ability to synchronise to light–dark cycles. These behavioural characteristics represent sensitive and novel metrics for assaying the suppression of mutant htt-dependent toxicity. We are also measuring visual tracking using the fly's optomotor responses, a simple measure of the fly's ability to maintain its orientation to a moving visual field. Our initial results suggest that this approach will reinforce and extend that from the anatomical analyses. In

summary, we have found novel behavioural phenotypes in HD model flies that will serve as sensitive metrics for validating genetic modifiers of mutant htt toxicity identified in yeast.

A.18 THE PATHOGENIC MECHANISM OF HUNTINGTON'S DISEASE-LIKE 2 MAY INVOLVE HAPLOINSUFFICIENCY OF JUNCTOPHILIN-3

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Background: Huntington's disease-like 2 (HDL2) is a progressive, late-onset autosomal dominant neurodegenerative disease that is clinically and pathologically very similar to Huntington's disease (HD). HDL2 is caused by a CTG/CAG expansion in junctophilin-3 (JPH3), a gene involved in the regulation of neuronal calcium flux. HDL2 patient brains show cortical and basal ganglia degeneration, with neuronal intranuclear protein inclusions resembling those detected in HD brain and intranuclear RNA foci containing the JPH3 transcript, similar to those observed in myotonic dystrophy. Overexpression of JPH3 exon 2A RNA with a long CUG repeat in cell culture leads to the formation of RNA inclusions and toxicity, further evidence that RNA toxicity may contribute to HDL2 pathogenesis. In addition, the sequestration of unprocessed JPH3 transcripts into foci suggests that perhaps less JPH3 transcript and/or protein are present in neurons, consistent with a loss-of-function model.
Aim: To determine if loss of JPH3 expression contributes to HDL2 pathogenesis.
Methods: We extracted RNA and protein from HDL2 postmortem brains and assessed JPH3 RNA and protein levels by real-time PCR and Western blot, respectively. We also examined the motor function of junctophilin-3 knockout mice.
Results: We detected a significant loss of JPH3 transcript and protein in the cortex of postmortem HDL2 brains, possibly because the unprocessed transcripts are sequestered into foci. Furthermore, analysis of the jph3 knockout mice revealed that the null model exhibits a progressive motor phenotype and the heterozygous model presents a milder phenotype.
Conclusions: The data reported here provide strong preliminary evidence that loss of JPH3 expression contributes to HDL2 pathogenesis. Taken together with our previous evidence that JPH3 with an expanded repeat results in the transcription of toxic RNA, we propose a model in which HDL2 pathogenesis is the result of both gain and loss-of-function mechanisms. This model provides insights into possible mechanisms that may be common among HD and HD-like disorders.

A.19 MOLECULAR APPROACHES TO DISSECTING THE PATHOGENESIS OF HUNTINGTON'S DISEASE

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Background: Huntington's disease (HD) is a fatal neurodegenerative disorder that is caused by an expansion of a polyglutamine (polyQ) tract in the protein huntingtin (htt), which leads to its aggregation in nuclear and cytoplasmic inclusion bodies. The misfolding of polyQ in htt into a toxic structure(s) is thought to underlie pathogenesis in HD.
Aims: Our laboratory utilises a broad array of molecular approaches in our efforts to understand how misfolded htt mediates pathogenesis, including structural analyses of misfolded proteins and their assembly into aggregates using atomic force microscopy, yeast genetic and chemical-genetic screens to identify proteins and small molecules that modulate aggregation and toxicity of mutant htt, and molecular genetic and pharmacological approaches in cellular and animal models of HD.
Results: We have recently made major progress in three areas. First, we have found that molecular chaperones may prevent toxicity of abnormal htt conformations by reducing levels of potentially toxic aggregation intermediates. Second, using genome-wide screening approaches in yeast, we have discovered novel sets of genes that are crucial for mutant htt toxicity. Finally, we have found that a reduction in the brain levels of the toxic kynurenine pathway (KP) metabolites 3-hydroxykynurenine and quinolinic acid ameliorates pathophysiology in an in-vivo model of HD.
Conclusions: Our results provide additional evidence that genetic screens in model organisms can successfully identify disease-modifying pathways that are conserved in lower and higher eukaryotes. Furthermore, our findings strongly implicate microglia and KP-mediated excitotoxicity in HD. Finally, the novel KP inhibitors we have generated may facilitate testing of the clinical relevance of this exitotoxic pathway in HD patients.