



EUROPEAN **HUNTINGTON'S DISEASE** NETWORK



**5<sup>TH</sup> ANNUAL PLENARY MEETING**

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## 5<sup>th</sup> Annual Plenary Meeting of the European Huntington's Disease Network

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## 5<sup>th</sup> Annual Plenary Meeting of the European Huntington's Disease Network

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The 5<sup>th</sup> Annual Plenary Meeting of the European Huntington's Disease Network (EHDN), held in conjunction with the 12<sup>th</sup> Bi-annual Meeting of the European Huntington Association (EHA), convened in Lisbon (Portugal) on September 5<sup>th</sup> and 6<sup>th</sup>, 2008. More than 500 participants from 16 European countries and elsewhere were welcomed by the Organising Committee, represented by the Chair of the EHDN, **Bernhard Landwehrmeyer** (Ulm, Germany), **Joaquim Ferreira** (Lisbon, Portugal) and **Beatrice De Schepper** (Moerbeke-Waas, Belgium). To encourage the participation of members from the Iberian Peninsula, simultaneous translation of the sessions into Portuguese and Spanish was provided.



"Huntington's disease" (HD) "is an orphan disease", said **Joaquim Ferreira**. "It is our mission to change this perception and to redirect public attention to HD. The EHDN is an organisation which can be copied by others as an excellent example of how this change can be done. Our goal is to find effective treatments for Huntington's disease and improve the quality of life of affected families." **Emilia Nunes** (Lisbon, Portugal), representing the Portuguese Health Minister Ana Jorge, told the audience that Huntington's disease has been included in the National Programme for Rare Diseases in Portugal, where the number of HD gene carriers is estimated as 1,100. Considering that Huntington's disease is a family disease, the total number of people affected is beyond all doubt much higher. The programme, which is running between 2004 and 2010, will allow gathering more accurate epidemiological data on HD. As the EHA president, **Beatrice De Schepper** highlighted the close collaboration between EHDN and HD families through different international and national HD lay associations. She drew attention to the concerns and needs of HD families towards our goal to stop and cure Huntington's disease, which requires joint efforts through a balanced cooperation between different disciplines and sharing of knowledge on the disease between experts and HD families. **Allan Tobin** (New York, USA), who was invited to chair the hot topic sessions, said that "this is the largest gathering of people who are interested specifically in Huntington's disease in the history of this field." As noted by **Bernhard Landwehrmeyer** in his welcome speech, "this is a sign of success for the Network."



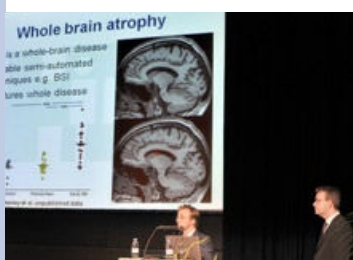
### Plenary Session I: Hot Topics

The first hot topic session was devoted to Huntington's disease pathology outside of the brain. **Gillian Bates** (London, UK) and **Maria Björkqvist** (Lund, Sweden) discussed recent research data providing evidence for signs of HD in non-CNS<sup>1</sup> tissues. Considering that the huntingtin (Htt) protein is produced in all tissues of the body throughout life, it is not surprising that, in mouse models, HD pathology can be detected in muscles, heart, pancreas, liver and other organs (e.g. in form of inclusion bodies). Addressing the question as to whether these mouse results are relevant and whether they are also seen in



<sup>1</sup> central nervous system.

humans, Bates and Björkqvist searched for this topic in the HD literature (approx. 8,000 articles) and found more than 500 publications referring to non-CNS symptoms or pathology in HD. Examples of peripheral manifestations of HD are weight loss, muscle atrophy, food dysphagia, gastrointestinal disturbances and mitochondrial dysfunction. The session was very interactive, with a lively participation from the audience. The discussion included topics such as weight loss (particularly in correlation with CAG repeat length, mitochondrial dysfunction, diet, rate of disease progression and mood), metabolism of muscle fibres and blood levels of cortisol. The later topic has been focus of research in many laboratories worldwide. For instance, a number of posters presented at the meeting reported high cortisol levels in the blood of HD patients, which can be already detected at pre-manifest and early HD stages. Another hot topic is the activation of inflammatory cells in HD, which is not restricted to microglial cells<sup>2</sup>, but also encompasses the peripheral immune system. A recently published study suggests that immune activation plays a role in brain pathology of HD, as increased levels of cytokines<sup>3</sup> are detectable in the blood of HD gene carriers even before symptom onset (see EHDN Newsletter Issue of December 2008 for a summary of this article). **Allan Tobin** closed the session with two open questions, which we should discuss further: 1) Is what we see in the periphery a reflection of what is going on in the CNS? and 2) Does a treatment for HD have to get into the brain or could it have beneficial effects by acting in the periphery?



The second session, presented by **Jan Kassubek** (Ulm, Germany) and **Edward Wild** (London, UK), focused on the use of imaging techniques to track disease progression in HD. Modern imaging techniques are being used to reveal HD pathology, to study its symptoms, to determine age at onset and progression rate, and to assess drug efficacy in clinical trials. Currently applied technologies include volumetric MRI<sup>4</sup>, functional MRI, voxel-based morphometry, brain parenchymal fraction, DTI<sup>5</sup>, MRS<sup>6</sup> and PET<sup>7</sup>. These techniques have been able to detect volume decreases in the striatum, caudate nucleus and cortex, as well as in the whole brain. This atrophy has been shown to correlate with CAG repeat length and disease stage. MRS helped find suitable creatine doses for clinical trials by measuring lactate concentrations, and PET showed decreased levels of dopamine receptors and microglial activation. PET has been also used to study Alzheimer's disease. Among others, these techniques offer the advantage of being non-invasive. However, they require expensive and sophisticated machines which are available only in specialised centres. In addition, movement disorders (e.g. chorea) make scanning difficult. There are also some open questions: For instance, it is unclear which the best imaging technique to study HD is, and how results from different studies could be compared. Maybe the best option would be to combine different techniques. Nevertheless, a central question remains whether we could trust an imaging-powered clinical trial, since drug efficacy per se has to be shown in disease manifestation (i.e. symptoms) in terms of improvement of functional performance in patients.

<sup>2</sup> immune cells of the CNS.

<sup>3</sup> key immune system molecules.

<sup>4</sup> magnetic resonance imaging.

<sup>5</sup> diffusion tensor imaging.

<sup>6</sup> magnetic resonance spectroscopy.

<sup>7</sup> positron emission tomography.



**Anne Rosser** (Cardiff, UK) and **Stephen Dunnett** (Cardiff, UK) gave an overview on cell replacement therapy as a means to replace CNS neurones – particularly medium spiny neurones (MSNs) of the striatum – which are lost in the course of HD. MSNs used for transplantation are taken from the embryonic striatum between the 8<sup>th</sup> and 10<sup>th</sup> week of gestation. Not only the appropriate phenotype (MSN), but also the right time point (i.e. developmental stage) is crucial for the success of the intervention. In rodent models of HD, behavioural improvement has been shown after grafting. “Does the procedure also work in humans?”, asked Rosser. A series of 40 neuronal transplants have been performed in HD patients within the French HD Network. Preliminary results are very promising, but a detailed characterisation of benefits and optimisation of the procedure will require subsequent studies. There are, however, some general limitations related to stem cell transplantation, in particular supply and demand, and achieving quality control. There is a pressing need for a renewable source of cells to replace human fetal tissue. These cells should be able to proliferate *in vitro*, to produce specific neuronal precursors and phenotypes, to integrate and reconstruct circuits, and to improve function. Various sorts of stem cells are likely candidates to fulfil these criteria. Two types of stem cells have attracted particular interest in this context: embryonic stem (ES) cells derived from the inner cell mass of blastocysts and tissue-specific stem cells (e.g. neural stem cells derived from fetal brains). ES cells will require the development of protocols to ‘direct’ them to an MSN phenotype, and although neural stem cells initially produce MSNs, with continued culture they lose positional information and stop producing this phenotype. Thus, a major research aim is to find ways of ‘persuading’ stem cells along specific phenotypic pathways. In order to facilitate this process, a key task is to develop a tool kit for reliably identifying MSNs in the culture dish. This is necessary because the protein DARPP-32<sup>8</sup>, which is used as a molecular marker of MSNs, is not consistently expressed in cell culture. A potential marker is FoxP1<sup>9</sup>, a protein belonging to the large Fox family of transcription factors, which seems to mark MSNs and co-labels with DARPP-32. In summary, the aim is to improve protocols for establishing stem cells in culture in order to achieve much greater proportion of DARPP-positive MSNs, eventually with systematic application of exogenous factors. Dunnett ended the presentation of this young and promising research field with open questions: 1) Is targeted (striatal) cell replacement relevant to HD?; 2) Are transplanted cells susceptible to host disease?; 3) Are symptomatic benefits sufficient or must we alter disease progression?; and finally 4) Are stem cells the way forward? The results from the first series of transplants in France, where according to **Anne-Catherine Bachoud-Lévi** (Créteil, France) three out of five patients showed improvements in functional ability, cognition and motor performance, give reasons for optimism.



When **Katharine Moser** (New York, USA) entered the stage, the audience was perhaps expecting a presentation on general strategies of how to cope with HD. Moser could have focused only on her experience as an occupational therapist caring for HD patients in a nursing home in Manhattan, but it was more than this. She came to tell us her own story: the story of HD in her life.

<sup>8</sup> dopamine- and cyclic AMP-regulated phosphoprotein with 32 kDa of molecular weight.

<sup>9</sup> forkhead box protein 1.

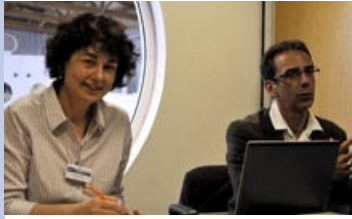
Moser comes from an HD family. Like her mother and her grandfather before, she carries the HD gene. In 2005, at the age of 23, Moser underwent predictive genetic testing for the HD gene. Of course, she hoped for different results, but she was also looking for answers. Her grandfather died with HD in 2002, after about two decades of being sick. For years, Moser saw signs and symptoms of HD in her mother. She knew that, if her genetic result was positive, then her mother definitely had the HD gene too. The genetic testing did not provide Moser with the answers she was looking for, but rather raised more questions about the future. "There is nothing more stressful than questions that don't have answers", said Moser, and she gets sad when she thinks about them. She gets sad when she thinks that we don't have a cure or an effective treatment for HD. It was even worse right after she tested.

"My issues of growing up in a family with HD", she said, "was not that my grandfather was sick, or that we were all at risk, or that my mother had passed the gene on to me. It was that nobody talked about it. My family never accepted that they were at risk. It was a secret and therefore scary." Moser realised that it was not the disease that was scary, but rather her family's denial. She mentioned the way how her patients cope with HD: "They allow Huntington's disease to be a part of their lives. Yes, they do have HD, but they still enjoy life."

Moser wants people to know that it is OK to talk about HD. She told her story to the New York Times, on TV and radio. She has met with politicians, companies, wealthy businessmen and students. "I search for any useful information on HD to remind my family and my friends that we are not alone", she said. Moser is indeed very committed in spreading information about HD and helping people better cope with the disease. In March 2007, the New York Times published an article about her entitled "facing life with a lethal gene". Thereafter, Moser received more than 600 e-mails. Some people told her that it was the first time they heard about HD outside their families. This stresses the need of building awareness about Huntington's disease, not only in the USA but worldwide, and of fostering research to find a cure.



Moser's story also raised many questions regarding predictive testing of a terminal disease without cure, in particular the challenges of living at risk facing lack of communication and denial, and the pros and cons of testing at such a young age. She closed her speech with two more unanswered questions: "How will you embrace Huntington's disease and how will you help us find a cure?" These questions were forwarded to the audience by **Bernhard Landwehrmeyer** (Ulm, Germany), who addressed especially healthcare professionals asking what they do to help affected people cope with HD. The feedback was very stirring, not only from physicians but also from researchers and lay organisation representatives. Moser did not fear to face her genetic heritage. People like her, who speak straightforwardly about HD and their feelings, inspire researchers all over the world to continue their efforts in searching a cure for HD.



## Plenary Session II: Working Group Summaries

This session was devoted to the EHDN Working Groups, which have been established with the aim to study particular aspects of HD. Hence, they are vital to the activities and success of the Network. There are now 18 working groups, 4 more than in September 2007. The new working groups are: 1) Biological Modifiers and Neuroprotection (led by Christian Neri and Gillian Bates), 2) Functional Ability (Aileen Ho and James Pollard), 3) Genetic Testing and Counselling (Gerry Evers-Kiebooms and Marina Frontali), and 4) Physiotherapy (Monica Busse and Lori Quinn).



## Session I: Working Group Sessions

The plenary session II started with short presentations of each working group by the respective lead facilitator focusing on overall goals, member profile, current and past projects, future aims and activities planned for 2008/2009. The audience had then the opportunity to learn more about selected working groups in extended oral presentations in the auditorium of the Pavilhão Atlântico. The working groups Imaging, Biological Modifiers and Neuroprotection, Biomarkers, Physiotherapy, and Surgical Approaches were presented in more detail. At the same time, participants could attend concurrent working group sessions in the form of convention centre-style booths, which provided current and future members with detailed information in the form of posters and handouts. People interested in joining a particular working group especially benefited from the informal exchange of ideas and one-on-one discussions.



The EHDN encourages its members to participate in a working group and thus actively contribute to HD research. To propagate the activities of the working groups and promote joining of new members, the EHDN Working Group Website (<http://www.euro-hd.net/html/network/groups>) has been recently updated and improved. Furthermore, the EHDN Newsletter (<http://www.euro-hd.net/html/network/communication/newsletter>) features two working groups per issue.

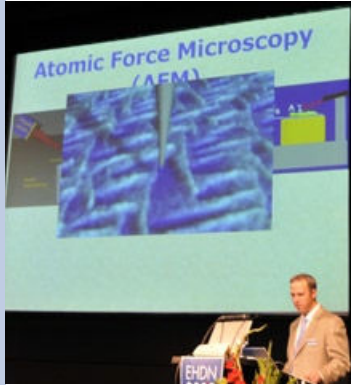
## Plenary Session III: Keynote Presentation

In the keynote presentation entitled "molecular approaches to dissecting the pathogenesis of HD", **Paul Muchowski** (San Francisco, USA) showed his results on putative molecular mechanisms of reducing huntingtin (Htt) neurotoxicity by preventing protein misfolding and aggregation.



Muchowski mentioned previous works by Max Perutz, Gillian Bates, Erich Wanker, Eberhard Scherzinger, Marian DiFiglia and others on the structure and function of glutamine repeats and their role in HD pathogenesis. Molecular modelling and X-ray diffraction studies of a synthetic polyglutamine (polyQ) previously showed that it forms beta-sheets held together by hydrogen bonds between the main chain and side chain amides. Glutamine repeats act as polar zippers promoting protein aggregation into amyloid-like fibrils. This self-aggregation is CAG repeat length dependent: It occurs only when the polyglutamine expansion is in the pathogenic range. Amyloid-like Htt aggregates (also called neuronal intranuclear inclusions) have been detected in brains of both transgenic HD mice and HD patients. According to the 'amyloid hypothesis', which has been primarily postulated for Alzheimer's

disease, protein aggregation is causally related to aberrant protein interactions that culminate in neuronal dysfunction and cell death.



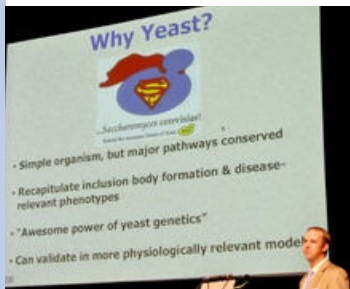
Using a novel technique, atomic force microscopy, Muchowski has studied the three-dimensional structure of Htt protein aggregates. This technique produces a 3D topographical image of any structure and can also be performed in solutions. Since some data from other brain diseases like Alzheimer's disease have suggested that oligomers – and not fibrils – were actually the molecules causing toxicity in neurones, the key questions of this study were: 1) What structures exist *in vivo*?; and 2) What toxic conformation or assembly mediates neurodegeneration *in vivo*? Muchowski demonstrated that, in solution, a recombinant mutant Htt protein fragment forms both structures: fibrils and oligomers (spherical and annular oligomers). He hypothesised that mutant Htt could fold in different monomeric conformations, which ultimately would form different types of aggregates, and that these molecules have differential toxicity to neurones. These hypotheses have the following implications for HD pathogenesis: 1) A mutant Htt fragment forms non-fibrillar oligomeric structures in a polyQ length-dependent manner; 2) PolyQ length and amino acid context determine the aggregate morphology and kinetics of oligomer formation; 3) These oligomers are not generally precursors of fibrils; and 4) Monoclonal antibodies can distinguish different polyQ conformations (monomers, oligomers and fibrils). These are important considerations for the therapeutic design of 'aggregation inhibitors' and intrabody-based gene therapy. The idea behind these clues is to generate an antibody which is able to bind specifically mutant Htt and clear it out of the brain, without affecting the normal (wild-type) Htt protein.

Similarly to Alzheimer's and other neurodegenerative diseases, HD is a protein misfolding disease, noted Muchowski. Hence, he applied a strategy based on the use of chaperones to correctly fold and to prevent misfolding of mutant Htt. Chaperones are proteins that assist the folding/unfolding and assembly/disassembly of other macromolecules (e.g. proteins). One major function of chaperones is to prevent proteins from aggregating into non-functional structures. Many chaperones are also heat shock proteins (Hsp) because the tendency to aggregate increases as proteins are denatured by heating. Chaperones have been shown to be neuroprotective in animal models of different polyQ diseases.

The paradox surrounding the neuroprotective function of chaperones is: If chaperones suppress aggregation, why are there still inclusion bodies in mice overexpressing chaperones? Muchowski postulated that chaperones are neuroprotective because they act on non-fibrillar Htt species, and that these assemblies cause neurodegeneration. By analysing the effects of the Hsp70 chaperone system on Htt aggregation, Muchowski demonstrated that, *in vitro*, the chaperone Hsp70 and its co-chaperone Hsp40 suppress the formation of oligomers and promote the formation of fibrils. Hence, they can modulate Htt aggregation. This process requires energy (ATP) supply. *In vivo* experiments showed that transgenic HD mice lacking both copies of Hsp70 had a worse performance in survival, rotarod and clasping analyses. Microscopy studies of the role of chaperones on protein aggregation revealed that deletion of Hsp70 increases both the density and the size (i.e. diameter) of inclusions.

Muchowski proposed a model for chaperone protection in that molecular chaperones are neuroprotective because of their ability to modulate the earliest aberrant protein interactions that trigger pathogenic cascades. The implication for HD is that Hsp70 plays a critical role in protecting against mutant Htt toxicity *in vivo*. The effect of Hsp70/Hsp40 may be based on the ability of these chaperones to shield toxic forms of polyQ-containing Htt fragments and to direct them into non-toxic aggregates. Thus, the potential usefulness of Hsp70/Hsp40 to suppress polyQ-induced neurodegeneration in HD should be further investigated. There are pharmaceutical companies interested in developing Hsp-inducer molecules to treat misfolding diseases. One example is the compound arimoclomol, which is currently being tested in a phase II clinical trial for treating amyotrophic lateral sclerosis.

Next, Muchowski introduced a new topic: the role of microglial cells in HD pathogenesis. It is well known that Htt is involved in many different processes within the cell. There is increasing evidence to suggest that mutant Htt impairs the ability of neurons to talk to each other, i.e. to form synapses. More recent studies have suggested that Htt can also affect other cell types, such as astrocytes<sup>10</sup>.



Using yeast cells, Muchowski showed that a mutant Htt fragment forms inclusion bodies in a polyQ length-dependent manner and is toxic in yeast. Starting from this observation, **Flaviano Giorgini** from Muchowski's lab screened for mutants that prevented Htt from killing yeast cells. This assay allowed the identification of 28 genes which suppressed Htt toxicity. The suppressor genes could be functionally grouped into four categories: 1) Protein transport and sorting, 2) Transcription, 3) Prion genes, and 4) Assorted processes and unknown function.

Muchowski then focused his further research on a single modifier gene of the above mentioned genetic screen: the kynurenine 3-monooxygenase (KMO) gene. KMO is a mitochondrial enzyme in the kynurenine pathway, where it catalyses the formation of 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN). These metabolites are highly toxic to the CNS. In the brain, KMO is expressed exclusively in microglia, the brain's resident immune cells. Previous studies performed by the late **Paolo Guidetti** showed increased levels of 3-HK and QUIN in vulnerable brain regions (striatum and cortex) of HD patients at early stages of disease. Muchowski postulated that, in microglia, Htt was turning this pathway on and thus contributing to neurodegeneration. Another reason for studying KMO was that the pharmaceutical industry has developed KMO inhibitors. One example is a compound called Ro 61-8048, which has been shown to be protective in models of brain ischemia and paroxysmal dyskinesia. However, this compound did not enter the brain very well.

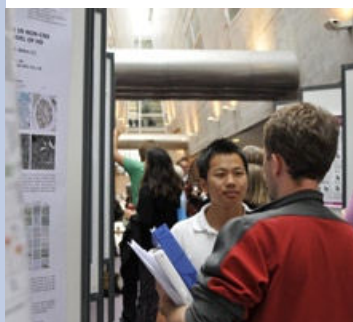
Together with his father Joseph, who is a medicinal chemist, Muchowski developed compounds chemically related to Ro 61-8048. One of them, JM6, was tested in transgenic HD mice in comparison to Ro 61-8048. The results showed that JM6 increased survival of R6/2 mice in a dose-dependent manner. It also decreased 3-HK and QUIN levels in the cortex and striatum. Im-

<sup>10</sup> star-shaped glial cells of the brain and spinal cord.

portantly, the cortex levels of QUIN negatively correlated with survival. Next, Muchowski studied the effect of the KMO inhibitor JM6 on microglial activation, a process that has been recently reported to occur at very early stages of HD. As revealed by immunohistochemical staining, immunoreactivity of microglial cells is reduced by JM6. The central question is whether JM6 confers neuroprotection. Looking at the level of synapses, Muchowski found that JM6 increases the striatal levels of synaptophysin, a protein used as synaptic marker. Interestingly, the synaptophysin levels negatively correlated with the levels of 3-HK and QUIN in striatum and cortex. A direct correlation between synaptophysin levels and survival of mice could also be detected. Similar results were observed using a marker for neuronal activity called c-Fos.

Currently, Muchowski is working on the characterisation of a knock-out *Kmo* mouse strain. Preliminary results have shown that partial deletion of *Kmo* seems to prolong survival of R6/2 mice. Concluding, Muchowski told the audience that mutant Htt can modulate the kynurenine pathway in microglia in a manner that contributes to pathogenesis in R6/2 mice. Besides suggesting an involvement of Htt in microglial dysfunction, his data also give additional support for the excitotoxicity theory and for the involvement of the cortex, in addition to the striatum, in HD pathogenesis.

### Session II: Poster Viewing



The first day of the meeting ended with a poster viewing session. More than 100 posters covering the following fields of HD were presented: pathogenic mechanisms (25 posters), experimental therapeutics / preclinical (5 posters), clinical characteristics (36 posters), biomarkers (13 posters), genetic aspects / testing (7 posters), clinical care and management (10 posters), and experimental therapeutics / clinical (5 posters). The abstracts of these posters were published in the *Journal of Neurology, Neurosurgery and Psychiatry* (BMJ Publishing Group), October 2008, Vol. 79, Supplement 1. A Committee composed of James Gusella (Boston, USA), Joaquim Ferreira (Lisbon, Portugal) and Hoa Nguyen (Tübingen, Germany) was nominated to judge and award the three most interesting posters. These were **poster C.30 by Ahmad Aziz** (Leiden, The Netherlands) showing that weight loss in HD is directly linked to CAG repeat length and is likely to result from a hypermetabolic state; **poster A.10 by Nicola Fearnley and Flaviano Giorgini** (Leicester, UK) on identification and characterisation of microglia-specific suppressors of mutant Htt toxicity; and **poster F.10 by LaVonne Goodman** (Lake Forest, USA), in conjunction with **Joseph Giuliano** (Princeton, USA) and **Debra Lovecky** (New York, USA), reporting the results of a survey of clinical trial interest, participation and literacy in Huntington support groups.

### Plenary Session IV: Presentation of Endorsed Projects

The second day of the meeting focused on research projects endorsed by the EHDN and scientific presentations. On behalf of the Scientific and Bioethics Advisory Committee (SBAC), **Anne Rosser** (Cardiff, UK) provided the audience with an overview of the application and review procedures of research projects. She reminded us of the aims of EHDN research: "Our priority is to facilitate and encourage high quality research in HD". Pursuing the final goal of finding a treatment for HD, the EHDN is providing an unparalleled collection of clinical data and biosamples, supporting research projects on

large scale, and building an infrastructure for large multi-centre clinical trials in Europe. There are three schemes through which EHDN members can apply for resources: data mining projects (clinical and biological data collected through REGISTRY), clinical trials, and seed fund applications (e.g. for working groups). Details of these schemes and the application procedure are given in the EHDN Newsletter Issue of September 2008, as well as on the website at <https://www.euro-hd.net/html/projects/proposals>. Since starting, 25 research projects were submitted: 21 are approved and going through the system, 3 are currently under revision and 1 is waiting for review. The majority requested data (21 projects), but there is also an increasing number of projects requesting genetic material (to date 8 projects). Project applications should be submitted electronically through the EHDN Website. Applicants must be regular or associated members of the EHDN and have a login to the EHDN web portal. The EHDN Central Coordination offers help with planning a project application. Applicants are encouraged to contact **Michael Orth** for advice.



On behalf of the Genetic Modifiers Working Group (GMWG), **Lesley Jones** (Cardiff, UK) presented a genome-wide association (GWA) study searching for genetic modifiers of HD. Given that the CAG repeat length determines only 50–70% of the age at onset, there are other modifying genetic factors that influence the onset and progress of HD. “Investigating these modifier factors gives you a direct line to those genes that may be affecting the onset of the disease. Therefore, they lie in pathways that are interesting as targets for disease-delaying therapies in HD”, said Jones. GWA studies aim to detect single nucleotide polymorphisms (SNPs) across the genome. One example of the usefulness of these studies for identifying genes involved in pathogenesis is the Wellcome Trust Case Control Consortium, which examined genetic modifiers of seven major diseases among the British population. Jones stressed the importance of the sample size to detect differences in HD age at onset. “The more samples you have, the more powerful the study. If you have 10,000 samples, you could detect genetic variation that gives a mean age at onset difference of around 12 months, which is quite good”, she said. Hence, increasing sample size enhances statistical power. It enables you to detect smaller effect sizes (measured as differences in age at onset), eliminate false positives and confirm true findings. The EHDN REGISTRY Project provides the samples for the genetic studies. There are currently approx. 3,800 subjects enrolled in REGISTRY. 2,600 biosamples have been collected and 1,700 DNA samples have been extracted (July 31<sup>st</sup>, 2008). The CHDI Foundation will genotype these samples on Affymetrix 1M SNP chips. These data will be provided to the GMWG to perform an analysis using age at onset as the major phenotype. Further analyses will follow taking into account relevant clinical data deposited in REGISTRY. To this end, the GMWG developed a ‘clinical characteristics questionnaire’, which will be implemented together with REGISTRY version 3. The information collected will be used to investigate whether the age at onset of a particular symptom is heritable, and what genes are associated with this symptom, as well as to design analyses with the appropriate sample size. This is important because, rather than a prior hypothesis, GWA studies need phenotypic data. The overall aims and current projects of the Genetic Modifiers Working Group are described in more detail in the EHDN Newsletter Issue of December 2008.



Another genome-wide association screen for Huntington's disease modifiers was presented by **James Gusella** (Boston, USA). "The initial targets", said Gusella, "are genes that modify age at neurologic onset, and the long-term goal is to identify modifiers of any phenotype with the underlying assumption that, if they modify *in vivo*, they automatically, by definition, affect the disease process. Therefore they would be ideal targets, already validated in humans, for developing therapeutics". The underlying principle is that "genetics provides an effective unbiased tool for discovering factors that trigger or modify disease, without any prior knowledge of the nature or mechanism". With 'genetics', Gusella means relating differences in DNA sequence (genotype) between individuals to differences in disease expression (phenotype). This is the tool applied originally to find the HD gene and which can now be used to identify modifier genes. "Linkage studies mapped the HD gene to chromosome 4, but in a broad area (about 2 million base pairs). Association studies narrowed it down to a specific gene. That's how we were able to locate the CAG repeat within the HD gene", said Gusella. He reported the HD-MAPS Project, a study of genetic modifiers of age at onset in pairs of siblings. A full genome linkage scan for regions with modifiers was performed using highly polymorphic microsatellite markers. The phenotype used in this study was the age at onset difference, i.e. the difference between the observed and the expected (according to CAG repeat length) age at neurologic onset. Analysis of approx. 1,200 independent samples of sibling pairs yielded evidence of modifiers with genome-wide significance values in a particular broad region (approx. 30 million base pairs) of the chromosome. For localising the HD gene, association with multi-allele markers was applied to identify the gene location more precisely within the region of linkage. Now, using the technology derived from the International HapMap Project, a genome-wide, public database to guide selection of efficient and powerful 'tag' SNPs for genetic association studies, it is possible to use the association strategy to look at all regions of the genome at once. Consequently, Gusella and collaborating colleagues from around the world are carrying out a GWA scan using Affymetrix v.6.0 SNP arrays, which are optimised to allow the detection of both SNPs and copy number variants (CNVs). The overall goal is to scan the genome for both SNPs and CNVs associated with altered age at neurologic onset of HD. The expected outcomes from this study are a set of significantly associated SNPs and common CNVs to be advanced to validation in additional samples. It will also produce a genome-wide dataset for testing association to variation in other HD phenotypes in the genotyped individuals. Gusella discussed issues related to cleaning of the data and population stratification (due to the fact that different populations have different genetic variations). The advantage of GWA studies is that they typically narrow the search to one or two gene candidates. The established genotype dataset is available for testing any heritable phenotype (e.g. pre-symptomatic, onset, manifestations, progression, response to treatment, etc). Concluding, Gusella highlighted the importance of sample size for statistical significance, as well as the need for high-quality phenotypic data. Therefore, a large-scale collaboration is crucial for the success of GWA studies.



**Vinayagam Arunachalam** (Berlin, Germany) from Erich Wanker's lab is also hunting for Huntington modifiers using a systems biology approach. Based on the results from the HD-MAPS Study, Arunachalam has examined SNPs

within the identified modifier loci that could explain the variation in HD age at onset. His approach applied a ranking of putative modifier, protein coding genes (modulators) according to their relevance to HD. Ranking was done by comparing functional datasets, like protein-protein interaction (PPI) network and gene expression data. A study done by Linda Kaltenbach from Robert Hughes' lab found genetic modifiers among proteins that directly interact with Htt<sup>11</sup>. This gave the hint for creating a PPI network specific for Huntingtin. Assuming that the HD modulators might have a border role in other neurodegenerative diseases, a larger PPI network was established which connects HD to other brain diseases (e.g. Parkinson's and Alzheimer's diseases). To narrow down the number of putative modifier genes, Arunachalam's study also assumed that the modulators might be differentially expressed in the brain, during ageing, and in HD patients. Approx. 1,800 genes were analysed according to this ranking model and yielded a final ranked gene list of 19 modifier genes. From the available literature, these genes are functionally involved in neurodegeneration, protein folding, apoptosis, immune response and metabolism. Next, the modifier genes in 22 Austrian HD patient samples were sequenced to detect SNPs. 343 mutations were identified and ranked according to significance. The Project intends to genotype 30–40 SNPs in 1,000 HD patient samples provided by the EHDN. Currently, 14 non-synonymous SNPs are being genotyped.



An interactive case vignette discussion session on management of HD was led by **Michael Orth** (Ulm, Germany). Two HD cases were presented by videos. Professionals from the audience who see HD patients were asked to answer anonymously a number of questions using remotes, with the answers being recorded by the host. The questions concerned the patient's most pressing problem and main symptom at onset, diagnostic confidence, management options, therapies chosen (pharmacological and non-pharmacological) and assessment methods of treatment effects. The answers could be broken down according to: a) profession of the rater; b) country where the rater works; and c) how many HD patients seen per year (reflecting experience of the rater). The results to each question were shown graphically in order to inform the audience. This device is ready to be used in national meetings, e.g. for rater training and certification. For further information, please contact Michael Orth at the EHDN Central Coordination.



### Plenary Session V: Business Meeting

The EHDN Executive Committee (EC) had proposed a number of changes to the EHDN Constitution, which were presented in detail by **Robi Blumenstein** (New York, USA) on behalf of the EC in the EHDN Newsletter Issue of June 2008 and summarised at the Meeting by **Raymund Roos** (Leiden, The Netherlands). The amendments concern the following Articles of the Constitution: 8) Review and endorsement of project applications – particularly the role of the EC and the SBAC (see EHDN Newsletter Issue of September 2008 and plenary session IV of this report); 9) Access to EHDN resources – especially clinical data and biomaterials donated by HD patients and collected through EHDN studies; 10) Publication and authorship; and 11) Ethics and conflicts-of-interest. The proposed changes were subject to consideration by the EHDN

<sup>11</sup> A summary of this research article by Diana Raffelsbauer was published in the EHDN Newsletter Issue of June 2008.

members, who had the opportunity to comment on them through the EHDN web portal for several months prior to the meeting, and by voting online from August 29<sup>th</sup>. The voting was closed at the Plenary Meeting in Lisbon, with the proposed amendments to the EHDN Constitution being approved by the vast majority of the members (103 of 116 people voted in favour).



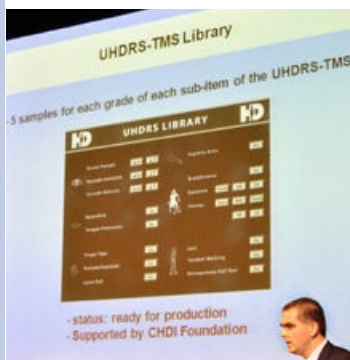
As the Chair of the EHDN EC, **Bernhard Landwehrmeyer** (Ulm, Germany) reported the activities of the Network between September 2007 and September 2008. He began by thanking **Justo García de Yébenes** (Madrid, Spain), who is rotating out of the EC, for his contribution to the EHDN. "Justo has done a fantastic job and has been extremely helpful and instrumental for us", said Landwehrmeyer. Justo García de Yébenes will be replaced by **Joaquim Ferreira** (Lisbon, Portugal), representing the Iberian Peninsula. In 2009, two further EC members will rotate out of office: Stefano Di Donato (Italy) and Jan Roth (Czech Republic). Hence, the EHDN welcomes candidates for these positions. Landwehrmeyer stressed that special attention will be given for regional representation. Therefore, it would be highly appreciated if EHDN members from Italy and Eastern European countries could make themselves available. Likewise, four SBAC members will rotate out of office. Members are welcome to make suggestions and nominate other people or themselves for these positions electronically via the EHDN web portal (<https://www.euro-hd.net/html/network/project/voting>).



Photo: Justo García de Yébenes

Landwehrmeyer summarised the progress of the Network within the last 12 months. "We have 694 regular members and 74 associated members", he said. This represents an increase of 250 regular members and 50 associated members over the past year. The Network currently operates in more than 120 study sites in 16 European countries, and an expansion into Russia is planned for 2009.

Since September 2007, the Network has organised a large number of regional meetings in each language area. "These are very important", said Landwehrmeyer, "because they provide a means of communication without a language barrier. They allow us to foster national, country-specific networks and network activities, which have already been particularly successful in the UK and Italy". The regional meetings can also be used to provide training sessions. Those already offered by the Network include training for motor, cognitive and behavioural raters. Landwehrmeyer briefly explained the procedure of motor rater training and certification, the EHDN UHDRS-TMS Certification, which will be mandatory for all motor raters as from 2009. An UHDRS teaching video and library will be available soon.



Plenary session II was designed to communicate the aims and achievements of the EHDN working groups to the wider membership and to attract members with knowledge and skills in the required specialist areas to participate. In the following year, the various working groups will sharpen their goals and update their working plans. "We are looking forward to face-to-face meetings of the respective working groups and are planning well prepared review sessions for lead facilitators, which will be organised by the Central Coordination", said Landwehrmeyer.

There are currently 3,798 participants in REGISTRY, which is an increase of 1,290 upon last year. The number of visits increased by 3,644 to 8,407 since September 2007. Landwehrmeyer highlighted the contribution of Leiden, Manchester and Madrid-Ramón y Cajal, which topped the list in terms of patient numbers. Biosamples were collected from 2,188 participants, an increase of about 1,000 since last year. The main contributor of biosamples has been the UK, followed by Germany and then Spain. At this point, Landwehrmeyer reminded the audience that the data in REGISTRY are available for research purposes, e.g. for data mining projects. The project application process, as presented by **Anne Rosser** in the plenary session IV, is very transparent, and help is available from the Central Coordination.

Next, Landwehrmeyer discussed the quality of the data in REGISTRY. The use of eCRF<sup>12</sup> allows in-built plausibility checks based on the analysis of rating scores. In addition, on-site monitoring by EHDN Language Coordinators ensures the verification of source data. Examples of judgement call problems were given, for instance recording age at onset, which is crucial for genetic modifier studies. In a small number of cases, a reassessment of the patient in view of all data available may be recommended. These instances of inconsistent data have highlighted the need to develop and improve questionnaires. An improved form designed to assess age at onset will be provided by the 'HD clinical characteristics questionnaire' mentioned by **Lesley Jones** in the plenary session IV.



The Network is currently working on a new version of REGISTRY (version 3). The scientific project manager is **Olivia Handley** (London, UK). The goal of REGISTRY 3 is to facilitate and accelerate the focused development and validation of additional explorative assessment tools. The statistical analysis of REGISTRY data collected using these potential new outcome measures will allow the statistical power of these tests to be determined. The end result will be an extended suite of assessment options that can be applied to all forms of HD, including pre-manifest, late stage and juvenile HD. Assessments will also be offered on an additional basis when specific rating scales are available that are applicable to particular HD symptoms (e.g. irritability or depression). The new explorative assessments will include the following aspects: functional ability, behaviour, cognition (extended form), physiotherapy, lifestyle and quality of life.

As aims for 2009, Landwehrmeyer mentioned that 1) The activities of the EHDN Working Groups should be disseminated in the form of publications; 2) REGISTRY 3 will be implemented; 3) The standard of care guidelines will be made available for all member countries via the web portal; 4) New web-based services for HD families and the general public will be offered; and 5) The EHDN Website will be improved to allow easier navigation. This will comprise, for instance, a new section with detailed information about HD, written by **Diana Raffelsbauer**.



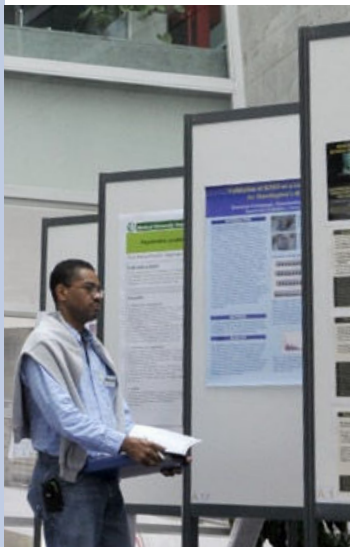
There will be no EHDN Plenary Meeting in 2009, but the World Congress on Huntington's disease will take place in Vancouver (Canada) from September 12<sup>th</sup> to 15<sup>th</sup>. As the future host, **Michael Hayden** (Vancouver, Canada) outlined the focus and programme of the congress. "We are hoping to make this

<sup>12</sup> electronic case report form.

meeting extremely interactive. The World Congress is a meeting for the entire Huntington's disease community, both the scientists and the families", said Hayden. Inputs into the planning of the meeting are very welcome. The aim is to integrate issues that are of concern to families and are also critical for scientists and physicians. If you have any suggestion or idea for the sessions, please contact Michael Hayden or Blair Leavitt. Registration will be open in January 2009 and the deadline for abstracts will be in May. For further information, please visit <http://www.worldcongress-hd.net/>.

### Plenary Session VI: Scientific Presentations

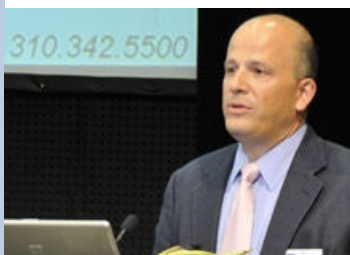
This session was devoted to scientific presentations. Selected researchers presented their most recent scientific results in the following fields and topics:



Pathogenic mechanisms		
Increased activity of the hypothalamic-adrenal-axis in early stage Huntington's disease patients	<b>Ahmad Aziz</b> (Leiden, The Netherlands)	Poster D.2
Disentangling molecular interaction networks for Chorea Huntington	<b>Matthias Futschik</b> (Berlin, Germany)	Poster A.1
Experimental therapeutics (preclinical)		
Genetic knock-down of HDAC4 improves motor impairment in the R6/2 mouse model of Huntington's disease	<b>Michal Mielcarek</b> (London, UK)	Poster B.5
Genetic aspects and testing		
Intermediate alleles for Huntington disease: patient understanding and current genetic counselling practices	<b>Alicia Semaka</b> (Vancouver, Canada)	Poster E.1
Huntington's disease and Huntington-like phenotype: 10 years of local molecular diagnostic experience	<b>Jorge Sequeiros</b> (Porto, Portugal)	Poster E.7
Clinical care and management		
The Huntington's patient quality of life project: an update	<b>Aileen Ho</b> (Reading, UK)	Poster F.3
Clinical characteristics and biomarkers		
Pain in Huntington's disease	<b>Marina de Tommaso</b> (Bari, Italy)	Poster C.18
Disturbed 'motor resonance' at the basis of the emotion recognition deficit in Huntington's disease? An EMG investigation	<b>Iris Trinkler</b> (Paris, France)	Poster C.13

### Plenary Session VII: Keynote Presentation

**Robert Pacifici** (Los Angeles, USA) gave a brilliant presentation on the Huntington's disease therapeutics programme developed by CHDI, a non-profit organisation that is pursuing a biotech approach to rapidly discover and develop drugs that prevent or slow the progression of Huntington's disease. Through collaboration with hundreds of academic and industrial scientists, CHDI supports a broad and diversified portfolio of programmes involving different organisms and platforms aiming to create the next generation of hypotheses for effective HD therapeutics. Some of these hypotheses are hopefully testable and robust enough to be demonstrated in Huntington's disease.



The methodology used by CHDI is based on a number of considerations for the drug discovery process, beginning with the choice of targets that are



compounds that have similar mechanisms, such as MAO<sup>13</sup> A inhibitors (e.g. moclobemide) and MAO B inhibitors (e.g. selegiline and rasagiline), unfortunately did not yield the expected results.

Reverse engineering is an approach that starts at the end of the drug pipeline and tracks drugs backwards from the clinic to the discovery process. A number of compounds, such as coenzyme Q<sub>10</sub> (CoQ), creatine and dimebon, are thought to work by rescuing energetic defects that are present in Huntington's disease. As these compounds move forwards in clinical development, the need for assay systems able to allow potency ranking of novel compounds arises. One example is a toxicity assay using HD patients' fibroblasts developed in collaboration with Edison Pharmaceuticals (San Jose, USA) based on results published by **Marcy McDonald** (Boston, USA) showing a decrease in ATP/ADP-ratios which are inversely proportional to the CAG repeat length. The hope is that these compounds can be improved, e.g. in terms of bioavailability or potency, leading to better activities in smaller, safer quantities over longer periods of time. Compared to CoQ, the Edison compounds are orders of magnitude more potent, showing that it is possible to develop drugs that have higher activity and can be used at lower concentrations.

A research field that has been focus of attention is the so called 'CoQ Mimicry', meaning the development of compounds that behave like coenzyme Q<sub>10</sub>. This requires redox cycling activity, i.e. the ability of the compound to transfer electrons and act as an antioxidant, thus reducing other molecules. A number of compounds developed by Edison were shown to function as CoQ mimics, as they were able to rescue CoQ deficiency in an assay with cells isolated from patients with a genetic defect impairing CoQ biosynthesis.



Drawing the attention to the peripheral effects of HD, Pacifici reported a study showing that lymphoblasts<sup>14</sup> derived from HD patients are more susceptible to oxidative stress, and that the Edison compounds were capable of rescuing the cells from oxidative insults *in vitro*. This provides a path to track drug (pseudo)efficacy *in vivo*. The pharmacokinetics/pharmacodynamic-relationship can be used to translate results from rodents to humans. In other words, if the compound gets into the mouse peripherally and centrally, and if it works both in the periphery and the brain, then you can easily check whether it works peripherally in humans and then extrapolate that probably it would also work in the human brain, explained Pacifici, "so it is a nice way of having a window to the central compartment by tracking activities in the blood."

Observational trials with a 'twist' examine HD patients not at rest but rather at bicycling and then measure a variety of factors, such as oxygen consumption, carbon dioxide expiration and different metabolites, including lactate, pyruvate, ketone bodies and 8-hydroxy-2'-deoxyguanosine (8-OH2'dG). To this end, CHDI designed a mitochondrial stress test with the hope to detect metabolic changes in HD. The test is currently being performed in 30 HD patients at stage 1 or 2, and the results are expected by mid-2009.

<sup>13</sup> monoamine oxidase (MAO) inhibitors, a class of antidepressant drugs.

<sup>14</sup> cells that differentiate to form lymphocytes (white blood cells).

Pacifici gave an overview of the various projects of the CHDI's portfolio, pointing to the diversity of their nature. They include, for instance, the inhibition of a number of key enzymes in different pathways (e.g. histone deacetylases, caspase-2, caspase-6, transglutaminase-2, KMO, JNK3<sup>15</sup> and PDE<sup>16</sup>), as well as Htt reduction and trophic factor agonism (e.g. BDNF<sup>17</sup>). The strategies that are being pursued comprise, among others, high-throughput screening, fragment screening, searching for existing ligands and rational structure-based design. Appropriate *in vitro* and *in vivo* systems have to be developed to test candidate drugs. Specific reagents and tools, either in biochemical or cell-based assays, are needed to prosecute a certain target. Likewise, *in vivo* systems have to be tailored specifically to each programme. Several considerations have to be taken into account, such as the genetic background of the animals and the types of pharmacodynamic measures to be used. "We are thinking not just about seeing efficacy within an HD model, but also whether the targets are relevant to a particular mechanism. If there is a mechanism that is relevant to humans, and that we can recapitulate and fix in rodents, then it may also be relevant to HD", noted Pacifici. The outcome measures of this 'mechanism-based (pseudo)efficacy' include cognition, metabolism, Htt fragment load and inflammation.

Pacifici discussed the strategies that CHDI pursues to design assays for *in vivo* testing. There are implemented standards for strains, genetics, husbandry, outcomes and data analysis which have proved to be a rapid way of screening through a large number of existing candidate compounds. He mentioned the long road of drug discovery that begins with the compound of interest and ends with an HD rodent model of drug efficacy, highlighting additional steps that CHDI have built in to improve this process. These include chemical synthesis leading to novel compounds, as well as pre-clinical formulation/pharmacokinetic studies to measure drug stability and exposure of the target. The maximum tolerated dose of a compound is determined to prevent efficacy being masked by toxicity. Broad target profiling screens the compound against a large panel of molecular targets *in vitro* aiming to correlate effect to target. Pharmacodynamics yields an elegant way of dose finding. CHDI is also studying quantitative and qualitative ways to improve the efficacy readouts, for instance by including other HD animal models/mouse strains, extending the list of collaborators and increasing the number of outcomes (motor performance, cognition, body weight, survival, etc).



Another approach to treat HD using RNA-based technology led to an antisense therapy project developed in conjunction with Isis Pharmaceuticals (Carlsbad, USA). The aim of this project is to identify an antisense oligonucleotide that reduces the levels of Htt in the brain. The antisense oligonucleotide needs to be modified and optimised for use in the CNS, taking into account that the middle gap of the molecule is important for binding to the sense RNA and degradation by RNase H. The candidate molecules are first tested in cell-based assays before advancing to *in vivo* pharmacology studies, which include analysis of Huntington's disease efficacy, central versus peripheral effects, route of administration and distribution (canine distribution study), and

<sup>15</sup> c-Jun N-terminal kinase 3, an enzyme found mainly in the brain.

<sup>16</sup> cyclic nucleotide phosphodiesterases involved in signal transduction.

<sup>17</sup> brain-derived neurotrophic factor.

on-mechanism toxicity to determine the therapeutic window. These aspects are crucial to find the effective dose beneath the toxic threshold.

Pacifici outlined preliminary results from an RNAi<sup>18</sup> study done in mice using an antibiotic-inducible shRNA<sup>19</sup> molecule against mouse Htt. The protein levels upon induction with doxycycline were reduced by more than 85%, which is very encouraging. However, there are some problems with this strategy, such as reduction of Htt even in the absence of the antibiotic (25% at RNA level and 50% at protein level). In addition, some adverse events were detected in the knock-down mice (e.g. weight loss, motor performance and behaviour). This exemplifies the complexity of determining how much of the Htt level can be reduced to yield a beneficial effect *in vivo*.

Concluding, Pacifici highlighted the role of CHDI in collaborative enablement of HD research. "We want to do everything that we can in terms of providing not just funding, but also whatever other resources are needed (domain knowledge, reagents, discovery skills, etc) for success in the drug discovery and development process. In the future, we will make things more transparent and easier. At the top of the list there is a more robust communication plan, led by **Bonnie Lee La Madeleine**. Without **Robi Blumenstein**, **Allan Tobin** and **Ethan Signer** this work would not be possible."

All in all, the EHDN 2008 Meeting was a successful and very interesting meeting covering different topics of HD basic and clinical research, and also psychosocial aspects of the disease. This event would not have been possible without **Bernhard Landwehrmeyer**, the **Local Organising Committee** (Joaquim Ferreira, Tiago Mestre, Ursula Kleibrink and Bea De Schepper) and the staff from the **EHDN Central Coordination** (Sonja Adam, Katrin Barth, Nicole Piller, Sonja Trautmann and others). With joint efforts from HD experts worldwide and the indispensable contribution of HD families, we hope to find a disease-modifying treatment for Huntington's disease in the near future.

**Imprint:**

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<sup>18</sup> RNA interference.

<sup>19</sup> small hairpin RNA.