Speaker Abstracts

Monoamine transporters as targets af CamKIIalpha: implications for Angelman syndrome
Harald H. Sitte, Ph.D. (Austria)

The nervous system is the prime organ for manifestations of Angelman Syndrom (AS). We have recently observed that the deficiency of Ube3A results in a change of dopamine transporter reactivity in the dopaminergic neurons (Steinkellner et al., J. Biol. Chem., 2012). Now, we examined the phenomenon that AS may often be accompanied by distinct side effects affecting the serotonergic system. These result mainly in an autistic symptomatology. Similar to the dopaminergic system, we have examined if the serotonin transporter, which is often involved in the manifestation of autistic symptomatology, and the serotonergic system *per se* poses a druggable clinical target. The serotonin transporter is well known as the target for a number of different drugs which affect several relevant disorders (e.g. depression). The lecture will summarize our current research and reveal an overview of the possibilities based on the involvement of the serotonergic system in AS.

Model Systems to study UBE3A/E6AP function
Ben Distel, Ph.D. (Netherlands)

The *UBE3A* gene encodes the ubiquitin protein ligase E6AP, for which impairment in E6AP-mediated ubiquitination of its target(s) is believed to cause Angelman Syndrome (AS). Although several potential targets of E6AP have been reported it remains to be demonstrated if any of these targets contribute to the AS phenotype. Therefore, identification of the (critical) E6AP target(s) and understanding their mechanistic contribution to the disorder is a first step in developing a therapy for AS. By employing yeast two-hybrid screens we have recently identified several high-confidence E6AP (UBE3A) interacting proteins (UIPs), including a protein known to cause intellectual disability with AS-like features, and a protein involved in vesicular trafficking in the brain. To determine if these proteins are *bona fide* targets of E6AP we have established the tools that allow us to 1) assess their direct physical interaction with E6AP and 2) determine how their ubiquitination and stability depends on E6AP activity. Here, I will present the validation of these tools and the initial characterization of the newly identified E6AP-interacting proteins. In addition, I will discuss how these tools can be employed to develop innovative approaches aimed at identifying novel neuronal substrates and activators of E6AP.

A critical role for UBE3A in brain development
Ype Elgersma, Ph.D. (Netherlands)

In this presentation I will give an update on our AS research lines. I will in particular focus on our data obtained from with inducible AS mouse model in which we can switch the UBE3A gene on at any desired time. Our experiments suggest that some, but not all, phenotypes of the AS mouse model are reversible when the gene is switched on in the postnatal animal. This indicates that there is a distinct critical window in which reversibility can be achieved, and that the critical window is not the same for all phenotypes.

Mechanisms of Ube3a Imprinting
Angela Mabb, Ph.D. (USA)

Angelman syndrome (AS) arises due to inherited deletions or mutations in the maternal copy of UBE3A. In neurons, the maternal copy of UBE3A is expressed but the paternal copy is silenced. One potential therapy for individuals with AS is to restore the paternal copy of UBE3A via pharmacological means. Using a high-content and high-throughput imaging assay, we identified that FDA-approved topoisomerase inhibitors unsilence the paternal copy of Ube3a in mice. This suggests a means to normalize UBE3A expression in individuals with AS and, hence, offers a unique therapeutic opportunity. We have sought to uncover the mechanisms of Ube3a unsilencing via direct manipulations in Topoisomerase through use of topoisomerase inhibitors, viral transduction, and transgenic mouse lines. Elaboration of these studies have given us a clearer view on how Topoisomerases regulate Ube3a silencing and advance our understanding regarding the impact of Topoisomerase manipulations during neurodevelopment. Our findings further aid in determining whether topoisomerase inhibitors are viable candidates for AS clinical trials.

Characterization of UBE3A mutations in Angelman patients
Silvia Russo, Ph.D (Italy)

Mutations and gross deletions within UBE3A gene cover about 10% of known defects involved in Angelman syndrome, a severe disorder characterized by mental retardation, peculiar EEG, absence of speech, ataxia, and a happy disposition. The gene encodes the E6-AP ubiquitin ligase and is subject to genomic imprinting with preferential maternal specific expression in brain and more specifically in neurons. On the other hand, maternal interstitial duplications of chromosome 15q11-q13 cause Autism. Out of a cohort of 163 AS patients with a genetic diagnosis we identified 41 different UBE3A mutations spanning the gene. Most sequence alterations are truncating, one is a splicing defect, 3 in frame deletions and 6 missense variant. In the effort to find out genotype phenotype correlation, nonsense mediated RNA decay in truncating mutations has been investigated, while predictions and

modelling of the missense mutations will be discussed. Mapping of missense mutations may be helpful in the discovery of crucial site in the protein.

News and views on UBE3A/E6AP and its role in ubiquitin conjugation
Martin Scheffner, Ph.D. (Germany)

UBE3A role in model systems of Angelman syndrome: regulation of protein homeostasis
Ugo Mayor, Ph.D. (Spain)

Regulated levels of the ubiquitin E3 ligase UBE3A are critical for appropriate brain development and function. Angelman Syndrome originates from lack of this protein in the brain. Which proteins are regulated by UBE3A in the brain is however still a matter of research. Using innovative technology developed in our lab for the isolation, identification and validation of ubiquitination substrates in the Drosophila model system we found direct ubiquitination by Ube3a of Rpn10, a proteasome-shuttling factor and three other proteins. Only Rpn10 is targeted for degradation upon ubiquitination by Ube3a, indicating that degradation might not be the only effect of Ube3a on its substrates. Furthermore, we report the genetic interaction in vivo between Ube3a and the C-terminal part of Rpn10. Overexpression of these proteins leads to an enhanced accumulation of ubiquitinated proteins, further supporting the biochemical evidence of interaction obtained in neuronal cells. Using our recently developed bioUb mouse, used here to characterize ubiquitination targets for a specific E3 ligase in the context of the whole organism, we now plan to further characterize the molecular mechanisms disrupted in Angelman Syndrome.

Using human induced pluripotent stem cells to model Angelman syndrome
Stormy J. Chamberlain (USA)

While much can and has been learned from mouse models of Angelman Syndrome (AS), there may be some differences between mouse and humans that can be missed by focusing solely on mouse models. We have generated induced pluripotent stem cells (iPSCs) from individuals with AS. iPSCs, which are functionally equivalent to human embryonic stem cells, can be converted into neurons. Thus, we can study AS in live human neurons that are growing in a dish. We are using our AS neurons for three major purposes: 1.) to understand how the neuron-specific imprinted expression of UBE3A is established, 2.) to understand the changes in neuronal gene expression that accompany AS, and 3.) to test potential therapies for AS.

Towards therapy for Angelman syndrome by knockdown of a repressive long non-coding RNA

Arthur L. Beaudet, M.D. (USA)

Angelman syndrome (AS) is a severe neurodevelpmental disorder caused by maternal deficiency of the imprinted gene UBE3A. Although the molecular mechanisms of maternal deficiency are diverse, all AS patients carry at least one copy of paternal UBE3A, which is silenced but intact. We aim to correct the expression level of UBE3A by activating the silenced paternal allele as a therapeutic treatment for AS.

UBE3A-ATS is the antisense transcript of UBE3A that negatively regulates its expression. By truncating this antisense RNA in mice, we showed that depletion of Ube3a-ATS is sufficient to activate expression of paternal Ube3a and rescue phenotypic defects in the AS mouse model, including motor defects, cognitive deficit, and impaired long-term potentiation.

To restore Ube3a levels in AS, we sought to activate paternal Ube3a by knockdown of Ube3a-ATS with antisense oligonucleotides (ASOs). With screens performed in cultured mouse neurons, we successfully identified mouse specific ASOs that fully activate paternal Ube3a. Unlike treatment with topoisomerase inhibitors, ASOs are site-specific and produce minimal effect on the nearby paternally expressed genes Snrpn, Snord115, and Snord116. We then tested the effect of ASOs in vivo by intracerebroventricular injection. Compared with PBS-treated animals, mice treated with ASOs show significant knockdown of Ube3a-ATS and activation of paternal Ube3a in the cortex and other brain regions four weeks post injection, indicating that the ASOs function effectively in vivo.

Current studies aim to provide additional mechanistic insight into the repressive function of Ube3a-ATS, achieve phenotypic improvement in the AS mouse model following CNS administration of ASOs, and identify human-specific ASOs for treating AS.

Targeted reaction of UBE3A in a mouse model of Angelman syndrome

David Segal, Ph.D. (USA)

Angelman syndrome is caused by loss of central nervous system expression of maternal UBE3A in neurons. Our goal is to reactivate paternal UBE3A, which is silenced due to the expression of the UBE3A-ATS antisense transcript. We are using zinc finger-based Artificial Transcription Factors (ATFs), which can be programmed to bind to a specific site in DNA. By attaching an activation or repression domain, the ATF can enhance or suppress transcriptional activity, respectively. A repressor ATFs designed to silence the Ube3a-ATS will be described that can be injected intraperitoneal as a purified protein, cross the blood brain barrier, and activate Ube3a expression in the neurons of a mouse model of Angelman syndrome. Our current progress and plans for future development of this approach will be discussed.

Clinical trial of Levodopa applied to Angelman Syndrome: objectives, methodology, assessment criterias and follow-up issues

Perrine Charles, M.D., Ph.D. (France)

Angelman syndrome is a neurodevelopmental genetic disorder characterised by severe learning difficulties, ataxia, early seizures (with a characteristic EEG), and happy social disposition without specific treatment to date. Animal models allow better understanding of the physiopathology of the disease. In Heterozygous mice with a maternally inherited Ube3a mutation who reproduce faithfully the human pathology:

- motor deficits could be attributed to dysfunction of the nigrostriatal pathway (Mulherkar et al, 2010)
- behavioral deficits correlate with abnormal dopamine signaling (decrease in dopamine release in the nigrostriatal pathway and increase dopamine release in the mesolimbic pathway) (Riday et al, 2012)
- misregulation of CaMKII is on of molecular causes for neurobehavioral deficits (Weeber et al, 2003)
- neurological deficits can be rescued by reduction of alphaCaMKII inhibitory phosphorylation (Van Woerden et al, 2007)
- Levodopa treatment, is also able to normalize alphaCaMKII autophosphorylation (Picconi et al, 2004).

Furthermore, Harbord had reported in 2001 Levodopa responsive Parkinsonism in 2 adults with Angelman Syndrome. Extra pyramidal features were not due to medications and were dramatically improved with levodopa therapy.

Those findings could lead to the development of new therapeutic perspectives in Angelman syndrome's care and management.